THE INCIDENCE OF ANADROMY AND SEASONAL ESTUARY USE OF NATIVE ATLANTIC SALMON AND BROOK CHARR, AND INVASIVE BROWN TROUT

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The Incidence of Anadromy and Seasonal Estuary Use of Native Atlantic Salmon and Brook Charr, and Invasive Brown Trout

by

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ABSTRACT

Brown trout were introduced to Newfoundland in the late 1800s and are now established in watersheds on the Avalon, Burin and Bonavista peninsulas. Little is known about estuary use by brown trout outside of their native or their impacts to native Atlantic salmon and brook charr. A relatively pristine watershed in eastern Newfoundland was chosen to investigate seasonal use of estuary habitat by these three species, and to investigated if 1) the incidence of anadromy in each species, 2) the quality of offspring of anadromous and resident, and 3) if the waterfalls are barriers to migration. A fourth objective was to test if a laser ablation "drilling" technique can successfully extract growth history information from otoliths. Abundance of adult brown trout was relatively consistent throughout the year, but capture of adult Atlantic salmon was limited to August (28:1 brown trout to salmon caught). Abundance of parr and smolt was greater in spring and summer, a pattern consistent between species, with the exception of a large pulse of salmon smolt during one day. Atlantic salmon and brown trout young of year were largely offspring of anadromous females, while brook charr young of year were largely offspring of resident females. Brook charr were bigger than Atlantic salmon at post emergence midstream, upstream and end of growing season upstream. Brown trout were bigger than Atlantic salmon at post emergence midstream, while the opposite was found at end of growing season downstream. No size differences were found between brown trout and brook charr. Otolith core Sr concentration was not a function of rearing habitat and laser ablation can clearly distinguish between freshwater and marine environments using a "drilling" or retrospective technique.

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Chapter 1: Introduction

Non-native species have had a profound effect on ecosystems around the world. In general, they are sometimes perceived as 1) beneficial, 2) harmful, and even 3) opportunities for scientific investigation of ecological and evolutionary processes (Inderjit 2005). The benefits of non-native species are often linked to global economies. The introduction, cultivation, and management of food crops (e.g., corn and wheat), livestock (e.g., sheep), as well as plant and tree species (e.g., eucalypts) that are grown outside of their native range have often been to great commercial advantage (Sax et al. 2007). These benefits are also associated with introduced fish and game species that have attracted many recreational users (Waters 1983).

However, many non-native species have proved harmful to their new environment, often to the detriment of native species. The intentional or accidental introduction of non-native species can lead to unexpected results, such as the uncontrolled dispersion into adjacent areas (i.e., invasion) (Pascual 2007). This unfortunate scenario has contributed to or caused changes to entire ecosystems and, in several cases, the extinction of several native species (Sax et al. 2007; Simon and Townsend 2003). Notable examples include the Asian longhorn beetle (*Anoplophora glabripennis*) in North America (Jenkins 2002), the Nile perch (*Lates niloticus*) in Lake Victoria in East Africa (Kaufman 1992), and zebra mussels (*Dreissena polymorpha*) in the Great Lakes in North America (Ricciardi 2003).

Non-native species have also provided scientists with unanticipated opportunities to better understand the natural world. They enable researchers to investigate ecological and evolutionary processes in "real-time" and across large temporal and spatial scales. Because the impacts of non-native species on new and pristine habitats has had serious and often negative effects on biodiversity worldwide (Clavero and Garcia-Berthou 2005), a great deal of effort has been devoted to investing these processes, determining which species are likely to be invasive, and resolving why potential invasive species succeed or fail. The term "invasive" is defined as per Lockwood et al. (2007); a species that is non-native (i.e., introduced to a novel environment through human actions) that spreads uncontrollably and that causes ecological damage. This body of research dates back to 1859 and Charles Darwin's influential work "*On the Origin of Species*". Charles Elton's "*The Ecology of Invasions by Animals and Plants*" (1958) has been essential in guiding much of the research on non-native species over the past 50 years. Specifically, Elton's diversity-invasibility hypothesis suggests that species-rich communities are less susceptible to invaders (Elton 1958; Richardson 2011). Furthermore, species-rich native communities frequently limits successful invasions, particularly if the previously established native species are competitively superior and share similar food and habitat requirements (Moyle and Light 1996). A recent example suggests that environmental resistance from abiotic factors (e.g., climate, habitat size, food resources and disturbance regimes) often limits successful invasions (Fausch et al. 2009). Finally, repeated introductions of a non-native species supplies continuous pressure on native species,

providing a numeric advantage (Fausch et al. 2009) and reducing allee effects, which may ultimately lead to successful invasions. This research has also provided important insights into why some species are likely to be invasive. For example, flexible life history strategies and the ability to rapidly adapt to new habitats has resulted in several salmonid fish species being labeled as successful invaders (Dunham et al. 2002; Jonsson and Jonsson 2011). Additional research suggests that understanding the niche characteristics of a particular species can help predict its invasion potential into new habitats (i.e., a match between species niche requirements in its native range and similar habitat availability in the new environment) (Korsu et al. 2007). However, despite these important insights, many questions remain as the ability to successfully invade varies among species (Jeschke and Strayer 2005) and is scale (Levine 2000) and context dependent (Fausch et al. 2009; Korsu et al. 2007). Further complicating matters, more recent work challenges some long-standing views established by Elton, further indicating the need for more research in this discipline; these works include "Invasion Ecology" (Lockwood et al. 2007), "Fifty Years of Invasion Ecology" (Richardson 2011) and "A Proposed Unified Framework for Biological Invasions" (Blackburn et al. 2011). These conclusions make it difficult for scientists to make recommendations to manage existing invasions and to predict future scenarios.

Brown trout (*Salmo trutta*) have provided scientists with numerous opportunities to study species introduction and invasion success. Brown trout is native to Eurasia and has been one of the most commonly introduced salmonid species; having now been successfully introduced nearly world-wide (MacCrimmon and Marshall 1968). Unfortunately, competition with native species has become of great concern, particularly in New Zealand (McDowall 2006), South America (Pascual 2007), and North America (van Zyll de Jong et al. 2004). Because of its impacts to native fishes, brown trout has the menacing distinction of being name one of the '100 worst invasive alien species' (of any taxa) by the Invasive Species Specialist Group (Lowe et al. 2000).

Brown trout on the island of Newfoundland provide a unique and important opportunity to investigate the successful invasion of an introduced species and the impacts on native salmonids. Brown trout were first introduced to the island of Newfoundland in the 19th century; the first individuals were introduced to systems in the vicinity of St. John's (Hustins 2007; Scott and Crossman 1964). These individuals were initially descendants of non-migratory (i.e., freshwater resident) parents (Hustins 2007); however, migratory populations are now common in Newfoundland watersheds (van Zyll de Jong et al. 2004). Recent work by Westley and Fleming (2011) shows that brown trout are now established in watersheds up to 500 km from the initial introduction site. It is thought that these systems were established by straying anadromous individuals (van Zyll de Jong et al. 2004; Westley and Fleming 2011), a pattern also documented in another brown trout invasion (Launey et al. 2010). Ironically, anadromy is often implicated in salmonid introductions that fail to establish self-sustaining populations outside of their native ranges; straying anadromous fish continue to stray rather than return to the same stream in successive years (Quinn 2005). However, in Newfoundland, anadromy and subsequent straying appears to provide a mechanism for continuing successful invasions.

In addition, most watersheds in Newfoundland have established populations of native Atlantic salmon and brook charr, which may present biotic resistance to invading brown trout (Moyle and Light 1996). Furthermore, most watersheds in Newfoundland are relatively pristine and free from anthropogenic disturbance (e.g., overfishing or logging) (DFO 2006), two characteristics that are not associated with successful invasions (Fausch 2008). Finally, brown trout were only introduced in the vicinity of St. Johns' and stocking efforts ceased in the early 20th century (Hustins 2007). This scenario provides a unique research opportunity, as brown trout in Newfoundland appear to be able to successfully invade relatively pristine systems, which may present biotic and environmental resistance, without being subsidized by repeated stocking events.

The introduction of non-native fish species has been acknowledged as one of the most significant threats to the persistence of native fishes (Wilcove et al. 1998). Newfoundland has most of the healthy Atlantic salmon populations in North America, as well as thousands of healthy brook charr populations, yet very little is known about established brown trout populations in Newfoundland and their impacts on native fishes. Recent work by Westley and Fleming (2011) investigated the distribution of brown trout in Newfoundland at the watershed scale; however, our knowledge of within stream occurrences is limited (Gibson and Cunjak 1986). Furthermore, whether brown trout establish resident populations or remain anadromous is unknown. An important observation from Westley and Fleming (2011) was that many of the watersheds that have been invaded by brown trout included estuary habitat; however, little is known of brown trout estuary habitat use outside of their native range, or of the potential for competition with and impacts to native Atlantic salmon and brook charr (Hustins 2007; Scott and Crossman 1964). In addition, our review yielded only one study, conducted in Denmark (where both Atlantic salmon and brown trout are native), which investigated estuary habitat use by Atlantic salmon and brown trout within the same watershed (Koed et al. 2006). Therefore, our knowledge of estuary habitat use by salmonids, and of what relative advantages there are to using estuary habitat, is still limited. Finally, there is a dearth of information comparing estuary habitat use by individuals at different life cycle stages (ie. parr, smolts and adults). Since both Atlantic salmon and brown trout are known to use estuary habitat, understanding how individuals at different life-cycle stages potentially interact is of great importance.With declines in populations of native Atlantic salmon and brook charr (DFO 2006) and patterns of species displacement that mirror those observed elsewhere (Korsu et al. 2007; Ohlund et al. 2008; Waters 1983), a better understanding of invasive brown trout is urgently needed for the conservation of native fishes.

The overarching goal of this study was to investigate several questions related to established brown trout populations and the subsequent impacts to native Atlantic salmon and brook charr populations. The Renews River watershed, a relatively pristine watershed, which was not originally stocked with brown trout, was chosen to investigate these questions. The questions were organized into the following two chapters (Chapters 2 and 3) and were prepared so that each chapter is a stand-alone article for the purpose of submission to scientific journals. In Chapter 2, three hypotheses were investigated: 1) the incidence of anadromy in each species is a function of distance to sea and barriers to

migration, 2) offspring of anadromous fish are of higher quality (i.e., bigger) than offspring of resident fish, and 3) waterfalls are barriers to migration and, therefore, brown trout should not be present upstream of these obstacles. A fourth objective was to test a laser "drilling" technique as a means of extracting information on growth history. The hypothesis was that the Sr concentration in the otolith core differs between offspring of anadromous and resident fish, and this can be detected by drilling. In Chapter 3, the seasonal use of estuary habitat by native Atlantic salmon and brook charr and introduced brown trout during parr, smolt and adult life-cycle stages was investigated. The hypothesis was that estuary use within a life-cycle stage will vary (*i*) seasonally, and (*ii*) between species.

The conservation of Newfoundland's native populations of Atlantic salmon and brook charr is important for sustaining biological diversity and both recreational and subsistence (i.e., Aboriginal fisheries in Labrador) fisheries. Furthermore, the investigation of brown trout in Newfoundland may provide new, important insights to our current understanding of why potential invasive species (i.e., species that are both impacting and spreading) succeed or fail; particularly since they appear to successfully invade systems that may present resistance. Finally, confirming laser "drilling" as an effective means of extracting information on growth history may allow for more complex and diverse studies, due to reduced handling and preparation requirements. The independent studies in Chapter 2 and 3 will contribute to these initiatives.

Co-authorship Statement

The author of this thesis planned, designed and carried out the data collection in the field and in the laboratory, processed and analyzed all the data, and wrote all the subsequent manuscripts. Dr. Craig Purchase and Dr. Geoff Veinott provided substantial contributions to experimental design and the evolution of data interpretation, as well as providing editorial reviews of all the chapters. Dr. Yolanda Wiersma provided similar contributions during committee meetings and reviews.

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Chapter 2: Distribution and incidence of anadromoy in invasive brown trout and native Atlantic salmon and brook charr in a relatively pristine watercourse in eastern Newfoundland

Abstract

Biological invasions have had a profound effect on biodiversity around the world. Brown trout were introduced to Newfoundland in the late 1800s and are now established in watersheds on the Avalon, Burin and Bonavista peninsulas, yet little is known about established populations and their impacts to native Atlantic salmon and brook charr. A relatively pristine watershed in eastern Newfoundland (not originally stocked with brown trout) was chosen to investigate if 1) the incidence of anadromy in each species is a function of distance to sea and barriers to migration, 2) offspring of anadromous fish are of higher quality (i.e., bigger) than offspring of resident fish, and 3) waterfalls are barriers to migration (brown trout should not be present upstream of these obstacles.) A fourth objective was to test if a laser ablation "drilling" technique can successfully extract growth history information from otoliths. The incidence of anadromy was not a function of distance to sea and barriers; Atlantic salmon and brown trout young of year were largely offspring of anadromous females, while brook charr young of year were largely offspring of resident females. Among-species size comparisons found brook charr were bigger than Atlantic salmon at post emergence midstream, upstream and end of growing season upstream. Brown trout were bigger than Atlantic salmon at post emergence midstream, while the opposite was found at end of growing season downstream. No size differences were found between brown trout and brook charr. Within-species comparisons were not possible because too few resident fish were detected. The two falls were not barriers to migration and species distribution patterns were analogous to those observed elsewhere in North America and Europe. Otolith core Sr88 concentration was not a function of rearing habitat and laser ablation can clearly distinguish between freshwater and marine environments using a "drilling" or retrospective technique.

Introduction

Biological invasions have had a profound and often negative effect on biodiversity around the world (Clavero and Garcia-Berthou 2005). The establishment of non-native species into new environments is relatively common, suggesting that these environments contain resources to support additional species (Sax et al. 2007). Much effort has been dedicated to determining which species are likely to be invasive and why potential invasive species succeed or fail (Fausch 2008; Kolar and Lodge 2001; Korsu et al. 2010; Moyle and Light 1996; Sax et al. 2007). However, it has been observed that the ability to successfully invade varies among species (Jeschke and Strayer 2005) and is scale (Levine 2000) and context dependent (Fausch et al. 2009; Korsu et al. 2007), making it difficult to predict future invasions.

Humans have long provided a mechanism for species invasions via intentional or unintentional introductions around the world (Lockwood et al. 2007). Species that are chosen for introductions often depend on human interest in them, as well as the distances from source populations (Fausch et al. 2009). Fish are often chosen for introduction to enhance freshwater fisheries throughout the world (van Zyll de Jong et al. 2004 2004). Because of their economic and societal value (eg. sport fishing and food source), salmonids are among the most frequently introduced fish species and are now established nearly worldwide (Rahel 2007). Ironically, native salmonids have declined throughout the world due in part to these introductions and subsequent invasions (Behnke 2002).
The establishment of introduced salmonids is influenced by environmental resistance, including abiotic and biotic factors, and repeated introductions (Moyle and Light 1996). Environmental resistance from abiotic factors, such as temperature, disturbance and flow regimes, stream size, and habitat factors correlated with stream gradient often limit successful establishment of invasive salmonids (Fausch et al. 2009). Environmental resistance from biotic factors, such as species-rich native communities, may limit successful establishment by an invader, especially if native species are competitively superior and share similar food and habitat requirements (Moyle and Light 1996). Alternatively, species-poor communities (Elton 1958), competitively inferior native species, as well as unexploited niche openings (Korsu et al. 2007), present limited biotic resistance and are often associated with successful invasions. Furthermore, habitat disturbance or alteration by human interference is also often associated with successful invasions by non-native salmonids (Elton 1958; Fausch 2008; Fausch et al. 2009). Finally, repeated introductions of a non-native species supplies continuous pressure on native species, providing a numeric advantage which may ultimately lead to successful invasions (Fausch et al. 2009).

Brown trout (*Salmo trutta*), which is native to Eurasia, is one of the most commonly introduced salmonid species and has been successfully introduced nearly world-wide (MacCrimmon and Marshall 1968). The earliest introductions occurred in 1852 in eastern Russia, followed by New Zealand in 1867 and North America in 1883 (Klemetsen et al. 2003). Following their introduction to numerous areas, competition with native species has become of great concern, particularly in New Zealand (McDowall

2006), South America (Pascual 2007), and North America (van Zyll de Jong et al. 2004). As a result of their impacts on native fishes, brown trout is listed as one of the '100 worst invasive alien species' (of any taxa) by the Invasive Species Specialist Group (Lowe et al. 2000). As there are no native salmonids in the southern hemisphere, in many areas it is fishes of the family Galaxidae that have been most affected.

In North America, competition between introduced brown trout and native populations of Atlantic salmon (*Salmo Salar*) and brook charr (*Salvelinus fontinalis*) is of particular distress. Atlantic salmon are native to both western Europe (where they co-evolved with brown trout) and eastern North America (where they co-evolved with brook charr) (Klemetsen et al. 2003). Competition between Atlantic salmon and brown trout has long been a concern (Fenderson 1954), as there is evidence to suggest that brown trout restrict Atlantic salmon from areas near river banks in larger streams (Heggenes et al. 1990). Brook charr did not co-evolve with brown trout and their densities are negatively affected by brown trout presence (Ohlund et al. 2008). In addition, displacement of native brook charr populations has been documented in Fausch & White (1981) and Waters (1983), where brown trout occupy the lower reaches of a river and brook charr occur further upstream and in headwater sections. One of the earliest brown trout introduction sites in North America is the island of Newfoundland. In 1883, the first individuals were introduced to systems in the vicinity of St. John's (Hustins 2007; Scott and Crossman 1964). These individuals were initially descendants of non-migratory (i.e., freshwater resident) parents (Hustins 2007); however, migratory populations are now common in Newfoundland watersheds (van Zyll de Jong et al. 2004). Recent work by Westley and Fleming (2011) shows that brown trout are now established in watersheds up to 500 km from the initial introduction site. It is thought that these systems were established by straying anadromous individuals (van Zyll de Jong et al. 2004; Westley and Fleming 2011), a pattern also documented in another brown trout invasion (Launey et al. 2010). However, ironically anadromy is often implicated in salmonid introductions that fail to establish self-sustaining populations outside of their native ranges; straying anadromous fish continue to stray rather than return to the same stream in successive years (Quinn 2005).

The establishment of invaders ultimately depends on successful reproduction and the survival of offspring that contribute to subsequent generations (Wood and Budy 2009). Survival of young-of-year salmonids often depends on occupying optimal feeding and overwintering habitat (Cunjak and Power 1986) and size of individuals often determines habitat use by brown trout, brook charr and Atlantic salmon (Harwood et al. 2002 2003). The positive relationship between adult body size and egg numbers/size suggests that larger females have a reproductive advantage (Morita and Takashima 1998). In temperate latitudes, marine waters are more productive than fresh waters and migrating to these prolific habitats often yields larger body sizes in migratory (anadromous) adults (Gross ; Tallman et al. 1996). Recent work by Jardine et al. (2008) found that relative abundance of offspring of anadromous females was greater in habitats where both anadromous and resident forms existed in sympatry; offspring of anadromous females were also larger than offspring of resident females at the end of the first growing season

following emergence. These findings suggest that offspring of anadromous females are afforded potential adaptive advantages during early stages of development, through the transfer of energy resources acquired at sea. However, potential anadromous parents are believed to have higher mortality rates associated with the challenges of migrating, including higher rates of natural predation and intense human fishing pressure where anglers disproportionately target anadromous fish because of their larger body size (Dieperink et al. 2002; Koed et al. 2006; Roche 1992). The development of anadromy is, therefore, suggested to be a trade-off between the increase in age-specific growth and fecundity and the reduced probability of reproduction because of the increased risk of mortality (Gross 1987). If invasive brown trout populations are predominately anadromous and produce higher quality offspring, this adaptive advantage may provide a mechanism for successful brown trout invasion into systems that may present environmental (pristine) and biotic (native salmonids) resistance.

The introduction of non-native fish species has been acknowledged as one of the most significant threats to the persistence of native fishes (Wilcove et al. 1998). Newfoundland has most of the healthy Atlantic salmon populations in North America, as well as thousands of healthy brook charr populations, yet very little is known about established brown trout populations in Newfoundland and their impacts to native fishes. Recent work by Westley and Fleming (2011) investigated the distribution of brown trout in Newfoundland at the watershed scale; however, our knowledge of within stream occurrences is limited (Gibson and Cunjak 1986b). Furthermore, whether brown trout establish resident populations or remain anadromous is unknown. With declines in populations of native Atlantic salmon and brook charr (DFO 2011) and patterns of species displacement that mirror those observed elsewhere (Korsu et al. 2007; Ohlund et al. 2008; Waters 1983), a better understanding of invasive brown trout is urgently needed for the conservation of native fishes.

The overarching objective of this study was to investigate several questions related to established salmonid populations in eastern Newfoundland. A relatively pristine watershed, where Atlantic salmon and brook charr are present and that was not originally stocked with brown trout, was chosen to investigate three hypotheses: 1) the incidence of anadromy in each species is a function of distance to sea and barriers to migration, 2) offspring of anadromous fish are of higher quality (i.e., bigger) than offspring of resident fish, and 3) waterfalls are barriers to migration and, therefore, brown trout should not be present upstream of these obstacles.

Distinguishing anadromous from freshwater resident fish

Despite the ecological significance between anadromous and freshwater resident fish, their identification has presented a major challenge using conventional techniques (Rieman et al. 1994; Theriault and Dodson 2003). The development of new technologies has enabled fish biologists to investigate questions of anadromy through the use of otolith microchemistry. Otoliths are calcium carbonate structures forming part of the inner ear of teleost fishes (Campana 1999). New material is continuously added to the otolith and

chemical elements from the surrounding environment are incorporated; therefore, the otolith contains a record of a fish's environmental use (Campana 1999). These chemical records have been used to investigate several questions relating to the movements and life-history events of fishes (Elsdon & Gillanders 2003; Elsdon et al. 2008). Strontium (Sr) concentrations are greater in marine waters and otolith Sr:Ca ratios have been used to identify migrations between marine and freshwaters in Arctic char (*Salvelinus alpinus*) (Babaluk et al. 1997; Radtke et al. 1998), brown trout (Limburg et al. 2001), Atlantic salmon (Friedland et al. 1998), rainbow trout (*Oncorhynchus mykiss*) (Zimmerman and Reeves 2000), and chinook salmon (*Oncorhynchus tshawytscha*) (Zimmerman et al. 2003). Adults captured in freshwater can thus be identified as being a previous marine migrator.

Transgenerational anadromy signatures can also be detected using otoliths. The source of elements in the otolith core is from the individual's mother by way of the yolk sac. Thus, influences from the maternally associated environment (in this case fresh or saltwater) are transferred to the offspring via its yolk (Volk et al. 2000). Otoliths are assumed to be a closed system, from the time eggs are spawned until they hatch (i.e., the otolith core is not influenced by freshwater chemistry post emergence); therefore, they can be used to identify individuals as the offspring of an anadromous or resident female (Kalish 1990; Rieman et al. 1994).

Standard techniques for analysis of Sr concentration in the otolith involve polishing or sectioning it to expose the core (Kalish 1990; Campana et al. 1997). This technique is labour intensive and may limit studies due to otolith preparation and handling time. Another technique, often used by palaeontologists to analyse the microchemical composition of rock and bone structures, is retrospective analysis, where a laser is used to ablate multiple layers of "growth" (i.e.,"drilling) (Mertz-Kraus et al. 2009). This technique is not widely used to analyse otoliths due to concerns over the accuracy and reliability of lengthy depth profiles (Chittaro et al. 2006). However, the drilling technique greatly reduces otolith preparation and processing times, which would allow for more individuals to be analysed and, thus, not limit studies of anadromy. A fourth objective was thus to test a laser "drilling" technique as a means of extracting information on growth history. The hypothesis was that the Sr concentration in the otolith core differs between offspring of anadromous and resident fish, and this can be detected by laser drilling.

Methods

Study Area

Sampling was conducted along the Renews River (46°55'N, 52°56'W), Newfoundland, Canada (Figure 2-1; inserted numbers are specific sampling locations, as referred to in the text). The main stem of the river is approximately 15 km long and the watershed includes numerous ponds and tributaries. Two falls (named First Falls and Second Falls) are present along the main stem and are assumed to be at least partial barriers to fish movement.

Fish Sampling

The sampling area consisted of a 10 km reach within the Renews River watershed, starting immediately upstream of the head of tide. Because the two falls were thought to be barriers to fish passage, the watershed was divided into three sections: *(1)* downstream of First Falls (DS), *(2)* midstream between Second Falls and First Falls (MS), and *(3)* upstream of Second Falls (US).

All fish were collected using a Smith-Root LR24 backpack electrofisher. Young of the year (YOY) Atlantic salmon, brook charr and brown trout were sampled from 16 sites (Figure 2-1, Appendix 2-A1) within these three sections during two seven-day sampling events in June and September 2009. Fish were collected during two distinct sampling events: 1) post emergence and 2) end of growing season. However, electrofishing is limited to between June 15 and September 15 of each year to avoid shocking fish during spawning and to protect fish eggs while incubating (i.e., pre emergence). Therefore, for the purposes of this study, post emergence and end of growing season are assumed to be June and September, respectively. Though emergence times and length of growing seasons can vary spatially and temporally, this assumption is generally supported by existing research (Klemetsen et al. 2003; Ohlund et al. 2008). Individuals were captured from multiple sites within each section and each sampling site consisted of a 100 - 200 m reach along the Renews River main stem or tributary. The goal was to collect between 30 – 60 YOY from each species within each river section; fish were collected from several sites within each section in order to ensure that individuals captured were not from the same brood (ie. siblings). Individuals were killed on site (using clove oil in accordance with Animal Care protocols) and placed on ice for transportation to the field office.

Upon arrival at the field office, fish were photographed against a white background, with a 10.0 megapixel digital camera. Fork-length for each individual (FL_U) was measured from the photographs using ImageJ 1.41™ to the nearest 0.1mm. Fish were then frozen and returned to the laboratory and stored at -10°C. Dry weights were measured following the removal of both otoliths and pectoral fins. Fish were placed in individually pre-weighed aluminum weigh-boats and inserted in a drying oven at 60°C for a minimum of 24 hours (which was determined to be long enough to bring them to constant weight), after which they were immediately weighed (DW_U to nearest 0.0001 gram).

Otolith Analysis

Upon extraction from the fish, otoliths were hydrated with several drops of distilled water to facilitate the removal of the adhering membrane and vestibule. Otoliths were rinsed again and allowed to air dry. The clean otoliths were then attached, sulcus side down, to glass slides using two sided tape and stored in sealed polypropylene containers. The otoliths were randomly arranged on the glass slides to ensure that analyses were un-biased with respect to the site or date they were collected. A total of 420 individuals were selected for otolith analysis, which included up to 30 random fish (if

captured) from each species, from each river section, from each sampling event (June & September).

Otoliths were analysed using laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS). Concentrations of strontium (Sr) 88 were determined using a Finnigan ELEMENT XR high resolution double focusing magnetic sector inductively coupled plasma mass spectrometer (HR-ICPMS) coupled to a GEOLAS 193 nm excimer laser system. A helium flow rate of 0.9 to 1.0 l/min was used to carry ablated material from the ablation cell to the ICP, with an additional 0.75 l/min argon make up gas added after the ablation cell. Stationary laser spots were used. A 60 μm laser beam was stationed over the otolith to produce depth profiles; from the top surface of the otolith into and through the otolith core. Laser energy was 3 J/cm^2 and the laser repetition rate was 10 Hz. Time resolved intensity data were acquired by peak-jumping in a combination of pulse-counting and analogue modes, depending on signal strength, with one point measured per mass peak. Analyses were carried out over 3 days and the ICP-MS was tuned each day for maximum sensitivity using the National Institute of Standards and Testing (NIST) standard reference material 612 (glass spiked with trace elements). Calcium was used as an internal standard to correct for differences in ablation yield. Approximately 30 seconds of gas background data were collected prior to each laser ablation of both standards and unknowns.

To determine the Sr concentration in the otoliths a data acquisition methodology of an analytical sequence of two analyses of the NIST 612 standard and one analysis of MACS1 reference material with analyses of up to 14 unknown otoliths, closing with a repetition of the same standards, was used. The NIST 612 standard was used to correct for instrument drift and changes in daily tuning. The MACS1 reference material has a similar matrix to the otoliths and was treated as an unknown. These data were acquired to allow the monitoring of accuracy and precision of the technique in general.

Data were reduced using Memorial University's in-house CONVERT and LAMTRACE spreadsheet programs, which employ procedures described by Longerich et al. (1996). Briefly, LAMTRACE compares the average Sr/Ca ratio in the NIST standard and the unknowns to the known Sr concentration in the standard to produce a Sr concentration in the unknowns. A Sr concentration for, approximately, each one second of ablation time was determined, which produced a concentration depth profile for each otolith.

To test whether a laser drilling method could detect an increased Sr signal in YOY otoliths from anadromous fish, brown trout with a known migratory history were analyzed. Young of the year brown trout from one female of known anadromous origin ($n = 15$) and one female of known resident origin ($n = 15$), were reared under controlled conditions at Memorial University's Ocean Sciences Centre. To insure that the entire otolith was core material, fish were killed immediately following emergence and prior to any feeding activities, then stored at −10 °C (Peter Westley pers. comm.). Otoliths were extracted and mounted on glass sides using the same methodology that was used with

YOY fish collected from the Renews River and described above. A fixed ablation signal interval of 30 – 90 microns depth was selected for determining the mean Sr concentration in each individual. This interval ensured there would be no contamination from the otolith surface or from the glue on the two-sided tape.

Testing for a transgenerational Sr signal in the Renews River YOY otoliths required comparing the otolith core chemistry to typical resident or freshwater otolith chemistry. The expected location (i.e., depth) of the core in the Renews otoliths was estimated based on the size of the otoliths reared under controlled conditions as well as the difference in size of the otoliths collected in Renews River in June and September. Once a core depth was estimated for the Renews sample a fixed ablation signal interval of 30 – 80 microns depth and 75 – 125 microns depth, was selected as the core for the June and September collections respectively. In order to assign individuals as offspring of an anadromous or resident mother, a known otolith Sr concentration for individuals that are resident to the Renews River was needed. Having already established the depth necessary to reach the otolith core, a fixed ablation signal interval of 6 – 12 microns depth was selected from 15 fish (chosen randomly) from each species captured in September. The ablation signal interval was selected from within the otolith edge to avoid contamination from the otolith surface and to ensure that no core material was included. Individuals captured in September were chosen in order to ensure adequate depth of freshwater growth. The average Sr concentration for each of the 15 fish was used to create a signal of "known" freshwater residence to the Renews River. This signal (N=15) was then used to compare to the core of each fish of unknown origin. This methodology is illustrated on Figure 2-2.

Water Sampling

To confirm that the transgenerational Sr signal was not influenced by water chemistry within the watershed, water samples were collected in June 2009 from eight of the 16 electrofishing sites (Appendix 2-A1) using trace clean plastic containers. Each container was rinsed three times before the sample was collected and nitrile gloves were worn to prevent contamination. Samples were acidified on site with 2ml of nitric acid (Veinott and Porter 2005) and kept cool (4°C) for transport to the laboratory. Samples were analysed by inductively coupled plasma-mass spectrometry (ICP-MS) and Sr:Ca ratios were determined.

Statistical Analysis

Statistics were conducted using the R statistical package (i.e., base package) version 2.10.0. Significance was set at $\alpha = 0.05$ and assumptions of parametric statistics were checked by examining model residuals.

Water Samples

The Sr concentration of the otolith core from five randomly chosen YOY of each species captured at each water sampling site was compared to the Sr concentration of each water sample site using a Pearson's product-momentum correlation test. Atlantic

salmon, brook charr and brown trout were captured at seven, eight, and five water sampling sites, respectively.

Size of Individual

Size metrics (fork-length and dry weight) of fish were analysed using Multivariate Analysis of Variance (MANOVA) ([FL_U] & [DW_U] ~ Species + River Section + Sampling Event + Species*River Section + Species*Sampling Event + River Section*Sampling Event + Species*River Section*Sampling Event). The dependent variables were fork-length and dry weight, while the independent variables were species (Atlantic salmon, brook charr and brown trout), river section (DS, MS and US) and sampling event (post emergence and end of growing season). Post-hoc Analysis of Variance (ANOVA) and Tukey analyses were used to determine fork-length and dry-weight differences among species within each river section during each sampling event.

Incidence of Anadromy

Using the control fish, the success of using a laser drilling method to detect a transgenerational Sr signal in the YOY otoliths of offspring from anadromous and resident females was determined using ANOVA ([Sr88] ~ Family), where 15 siblings provided the independent observations.

A transgenerational Sr signal in the otoliths of offspring from anadromous and resident fish captured from the Renews River sampling sites was determined using an ANOVA ([Sr88] ~ Individual). Analyses were completed for individuals of each species (Atlantic salmon, brook charr and brown trout) in each sampling event (post emergence and end of growing season) in each river section (DS, MS and US). Tukey post-hoc analyses were used to identify individuals statistically different from the "known" Renews River resident Sr signal. Individuals were assumed to be offspring of an anadromous mother if the Sr signal was significantly greater ($p < 0.05$) than the "known" Renews River freshwater resident Sr signal, and offspring of a resident mother if the concentration was not significantly different.

Results

Species distribution patterns

A total of 705 YOY salmonids were captured, recorded and retained from the Renews River watershed; Atlantic salmon ($n = 325$), brook charr ($n = 153$) and brown trout ($n = 227$). Table 2-1 shows the relative proportions sampled and kept for each species by river section and sampling event. See Appendix 2-A (Table 2-A1) for a summary of these data for specific sampling sites. Importantly, the ratios of species differences collected are conservatively biased. The goal was to collect between 30 – 60 individuals from each species in each river section; if enough fish from species "A" were collected, additional effort was used to try and get this sample size for species "B and C", but unfortunately extra individuals captured for species "A" were not recorded.

Abundances of fish of each species for a particular site, thus, vary more than the catch indicates, but it is impossible to determine by what magnitude.

All three species were captured in all three river sections, including upstream of both falls. Brown trout were captured at most sites along the Renews River main-stem; however, none were observed or captured at any of the tributary sites. Brown trout were more abundant in the DS and MS sections of the watershed. In the US section, more brown trout were captured in September than June (17 vs 1), despite similar effort in all three river sections. Atlantic salmon abundance was relatively evenly distributed throughout the watershed as fish were observed and captured at all sites, except for two tributary sites in the US section (site 14 and 15). Brook charr were observed and collected at most sites, including all US tributary sample sites; however, they were much less abundant DS.

Size of offspring

Although not dramatic, there were size differences in YOY fish (Table 2-1 and Figure 2-3). Analysis using MANOVA produced significant interaction terms; therefore, post-hoc ANOVA and Tukey analyses were run for fork-length and dry-weight. In both cases, the interaction term River Section*Sampling Event was significant; therefore, post-hoc ANOVA and Tukey analyses were run for forth-length and dry-weight during each sampling event (post emergence and end of growing season). In many cases, the interaction term Species*River Section was significant; therefore, further post-hoc ANOVA and Tukey analyses were run for each river section (DS, MS and US). Brook charr were significantly longer than Atlantic salmon at post emergence MS by 6.83 % ($F_{[2,124]} = 3.93$ $p = 0.0222$; Tukey $p = 0.0179$), US by 5.61 % ($F_{[2,114]} = 5.27$, $p = 0.00648$; Tukey $p = 0.0144$), and end of growing season US by 7.8 % ($F_{[2,125]} = 5.60$ $p = 0.00469$; Tukey $p = 0.0031054$), and were significantly heavier at post emergence MS by 31.27 % ($F_{[2,124]} = 5.30$ $p = 0.00621$; Tukey $p = 0.0234$). Brown trout were significantly heavier than Atlantic salmon at post emergence MS by 32.96 % ($F_{[2,124]} = 5.30$ $p = 0.00621$; Tukey $p = 0.0178$), while Atlantic salmon were significantly heavier than brown trout at end of growing season DS ($F_{[2,112]} = 7.72$ $p = 0.000723$; Tukey $p = 0.000465$) by 23.53 %. No size differences were found between brown trout and brook charr.

Incidence of anadromy

The Sr concentration in the otolith core of hatchery controls from a known anadromous mother (mean of 1633 ± 178 ppm SD) and resident mother (mean of 701 ± 114 ppm SD) differed among families (anadromous vs. resident) (ANOVA, $F_{[1,29]} = 1351.81$, $p < 0.001$), indicating the drilling technique could reliably detect a transgenerational marine Sr signature (Figure 2-4).

There was significant variability in the Sr concentration of the wild otolith cores among all three species. Tukey post-hoc analyses determined which individuals differed from the "known" Renews River resident Sr signal for each species. Table 2-2 summarizes the number of offspring of anadromous and resident fish. Atlantic salmon

and brown trout YOY were largely offspring of anadromous females, while brook charr YOY were largely offspring of resident females.

Water Chemistry

The Sr concentration in YOY otolith cores was not influenced by the freshwater chemistry in the Renews River. Pearson's product-momentum correlation test for Atlantic salmon (t = -1.424, df = 5, p = 0.214, cor = -0.537), brook charr (t = 0.131, df = 6, p = 0.900, cor = 0.0534) or brown trout (t = -1.852, df = 3, p = 0.161, cor = -0.730) indicate no correlation between otolith core Sr concentration and water samples collected from various sites along the Renews River for all three species.

Discussion

Results from this study suggest that brown trout have successfully invaded the Renews River watershed, which is relatively pristine, free from major anthropogenic disturbance and which was not originally stocked with brown trout. Within river species distribution patterns were similar to those observed elsewhere in North American and Europe; Atlantic salmon were present throughout most of the watershed, whereas brown trout were more abundant in downstream and midstream habitats and brook charr were more abundant in upstream and tributary habitats. The incidence of anadromy was not a function of distance to sea and barriers to migration. Atlantic salmon and brown trout YOY were largely offspring of anadromous females, while brook charr YOY were largely offspring of resident females; a pattern that was consistent throughout most of the Renews River watershed. However, two YOY brown trout offspring of a resident fish were captured (one in MS and one in US); evidence that some invading brown trout have adopted a non-migratory (resident) strategy in this watershed. Not enough fish were captured to make within-species size comparisons between YOY offspring of anadromous and resident mothers; however, among-species size comparisons were analyzed. Brook charr were significantly bigger than Atlantic salmon at post emergence MS, US and end of growing season US (too few brook charr captured DS to make a meaningful comparison). Brown trout were significantly heavier than Atlantic salmon at post emergence MS, while the opposite was found at end of growing season DS. No size differences were found between brown trout and brook charr. The two falls were not complete barriers to migration as brown trout and anadromous fish from all three species were captured above both falls. Lastly, there was an observed negative trend in fish size (both fork-length and dry-weight) from downstream to upstream.

Interestingly, these brown trout have successfully invaded the Renews River in spite of potential resistance. This watershed has two falls and established native populations of Atlantic salmon and brook charr, which may present biotic and environmental resistance to invading brown trout (Moyle & Light 1996). This watershed is also relatively pristine, with little known anthropogenic disturbance; a characteristic not normally associated with successful invasions (Fausch et al. 2009). Finally, the Renews River watershed was not originally stocked with brown trout, and stocking efforts in the vicinity of St John's (approx. 90 km away) ceased in the early 20th century (Hustins

2007); removing continuous pressure on native species and nullifying a numeric advantage. In spite of these potential resistance factors, brown trout have successfully invaded and are now present throughout much of the Renews River watershed.

Within river occurrences found Atlantic salmon present throughout most of the watershed, while brown trout were more abundant in downstream and midstream habitats and brook charr were more abundant in upstream and tributary habitats; a pattern similar to other systems in North America and Europe. Atlantic salmon are native to both western Europe (where they co-evolved with brown trout) and eastern North America (where they co-evolved with brook charr) (Klemetsen et al. 2003). Within a given system, these species select different habitats, but with considerable overlap (Heggenes et al. 2002). Habitat partitioning and co-evolution have provided time and opportunity for Atlantic salmon to establish co-existing life history strategies with brown trout (Klemetsen et al. 2003) and brook charr (Mookerji et al. 2004). Brown trout and brook charr did not co-evolve and interactions between these two species are the result of introductions to each fish's native habitat. Recent research suggests that niche characteristics are responsible for the patterns of brook charr and brown trout species distribution, whereby brook charr colonize niche space (upstream headwater habitats) only marginally used by brown trout, whereas brown trout colonized larger, more benign habitats further downstream (Korsu et al. 2007). A match between species niche requirements in its native range and habitat availability in the new environment may provide the basis for understanding invasion success (Korsu et al. 2010). Guillemette et al. (2011) found a positive relationship between the selection of spawning habitat and individual fitness in brook charr. These findings provide support for research by Ohlund et al. (2008) that proposed increased population fecundity in brook charr populations (expressed through both higher length-specific fecundity and higher proportions of mature females), rather than individual interference or exploitation competitive ability, limited competitive abilities and distribution of brown trout in brook charr dominated headwaters. Results from our study suggest that brown trout are indeed exploiting niche habitat in the river main stem and in downstream locations, while brook charr have retreated to their niche habitat in upstream and tributary locations.

The incidence of anadromy was not a function of distance to sea or barriers to migration. Atlantic salmon and brown trout YOY were largely offspring of anadromous females, while brook charr YOY were largely offspring of resident females; a pattern that was consistent throughout most of the Renews River watershed. The adaptive reasons for anadromy in salmonids almost certainly relate to more abundant food resources in marine habitats and the requirement for freshwater incubation environments (Gross et al. 1988). However, anadromy is suggested to be a trade-off between the increase in age-specific growth and fecundity and the reduced probability of reproduction because of the increased risk of mortality (Gross 1987). Although the two falls were not complete barriers to fish movement, the incidence of anadromy differed between these species. Brook charr are said to be less well adapted to marine life than other salmonids (McCormick 1994), which may result in fewer individuals adopting a migratory strategy, or fewer individuals successfully returning to spawn as a result of higher mortality rates

due to predation. Brook charr are also said to be more susceptible to angling pressure (Marshall and Maccrimmon 1970), which may also account for the observed ratios in this study. Anadromy is well documented in Atlantic salmon and brown trout and migratory populations are common throughout their habitat ranges (Jonsson and Jonsson 2009b; Klemetsen et al. 2003). One of the few studies on Newfoundland brown trout reported short marine migrations (typically less than 50 km) by anadromous fish (O'Connell 1982). Furthermore, brown trout use of estuaries for rearing and feeding is well documented in Europe (Jonsson and Jonsson 2009a, b; Knutsen et al. 2001; Rikardsen et al. 2006) and has recently been confirmed within the Renews River watershed (Chapter 3). These findings suggest that anadromous brown trout have adopted a migratory strategy to move only far enough towards the sea to take advantage of the benefits without undertaking a strenuous and potentially hazardous migration to fully marine habitats (Etheridge et al. 2008). In addition, the finding that YOY brown trout are largely offspring of anadromous fish, a pattern also documented in another brown trout invasion (Launey et al. 2010), provides further evidence that brown trout in Newfoundland appear to be an exception to the general paradigm of invasions. Anadromy is often implicated in salmonid introductions that fail to establish self-sustaining populations outside of their native ranges; straying anadromous fish continue to stray rather than return to the same stream in successive years (Quinn 2005). However, brown trout in Newfoundland utilize anadromy and subsequent straying as a mechanism for continuing successful invasions.

Our results yielded a small number of YOY offspring of resident Atlantic salmon ($n = 5$) and brown trout ($n = 2$). The occurrence of anadromous and resident Atlantic salmon is widespread in Newfoundland and the two forms are often sympatric (Scott and Crossman 1964; Verspoor and Cole 1989). The first brown trout introduced to systems in the vicinity of St. John's were initially descendants of non-migratory (i.e., freshwater resident) parents (Hustins 2007; Scott and Crossman 1964); however, migratory populations are now common in Newfoundland watersheds (van Zyll de Jong et al. 2004). Our finding of brown trout YOY offspring of resident fish is the first documented occurrence that we know of that shows that migratory or straying fish have adopted a non-migratory (resident) strategy in adjacent systems. These findings may provide evidence for distance to sea influencing the decision to migrate, or it may be the result of the availability of potential overwintering habitat in the mid-stream and upstream river sections.

Among-species size comparisons (not enough fish were captured to make within-species size comparisons between YOY offspring of anadromous and resident mothers) found that brook charr were longer (post emergence MS, US and end of growing season US) and heavier (post emergence MS) than Atlantic salmon, brown trout were heavier (post emergence MS) than Atlantic salmon, and Atlantic salmon were heavier than brown trout (end of growing season DS). No size differences were found between brown trout and brook charr. Fish size is important in determining habitat use by brown trout, brook charr and Atlantic salmon, and impacts of interactions between these species are thought to be highest during the first year of life (including first growing season and winter) when density-dependent processes are most intense (Milner et al. 2003). Both brown trout and

brook charr (both 0+ and 1+) prefer cover during winter, but brown trout occupy deeper areas than brook charr (Cunjak and Power 1986). Fish size also determines the use of over-wintering shelters (Harwood et al. 2002; Orpwood et al. 2003). Brook charr were larger (in both fork-length and dry-weight) than Atlantic salmon at post emergence MS and US and this may have helped them establish themselves in preferred midstream and upstream habitats following emergence. Their larger size at post emergence may also have provided them with an advantage through the first growing season as they continued to be larger (fork-length) than Atlantic salmon in upstream habitats at the end of growing season. Brown trout were larger (dry weight) at post emergence MS and Atlantic salmon were larger (dry-weight) than brown trout at end of growing season DS, which also may have helped them establish themselves in preferred midstream habitats following emergence.

The observed ratios of YOY offspring of anadromous and resident fish, coupled with among-species sizes, raises an interesting questions regarding the assumed benefits of anadromy and if this provides Newfoundland brown trout with a mechanism for successful invasions into systems that may present environmental (pristine) and biotic (native salmonids) resistance. Marine waters off the eastern coast of Newfoundland are more productive than its fresh water systems, suggesting that individuals adopting a migratory strategy will attain larger body sizes than non-migratory fish (Gross 1987). Recently, brown trout captured in the Renews estuary where shown to have higher growth rates than those captured in the river (Veinott 2009); this comparison can be made since brown trout captured in the Renews estuary are from the Renews River population (Veinott et al. 2012). Because of the positive relationship between body size and fecundity, anadromous females should have a reproductive advantage of increased fecundity and larger eggs (Morita and Takashima 1998). Our results suggest that, when compared among species, this is not the case. Atlantic salmon and brown trout YOY were largely offspring of anadromous females, while brook charr YOY were largely offspring of resident females. Yet, where size differences were significant, brook charr were larger than Atlantic salmon and no significant size differences were found between brook charr and brown trout. We must ask why brook charr offspring (largely offspring of resident fish) are as large as or larger than Atlantic salmon and brown trout offspring (largely offspring of anadromous fish). Research by Hutchings (1991) reported that brook charr females selected for larger egg size in resource limited (upstream and headwater) habitats, while Ohlund et al. (2008) found that brook charr had higher growth rates than brown trout in similar habitats. We did not investigate egg quality (numbers or size) in this study; however results from these studies may provide an explanation for our findings. Spawning and emergence timing also influence size of YOY, both following emergence and at the end of the first growth season. Early emergence is thought to provide an advantage in selecting territories, as a prior resident effect is well known in salmonids (O'Connor et al. 2000). However, early emergent fry may experience poor environmental conditions and an increased risk of predation (Brannas 1995). Early emergent fish are postulated to attain larger sizes at the end of the first growing season; however, evidence for this was found to be ambiguous and context dependent (Bagliniere et al. 1994; Egglishaw and Shackley 1977; Gibson and Cunjak 1986a).

Our study represents the first detailed investigation of within-river occurrences of Newfoundland's introduced brown trout. Our results show that species distribution patterns are similar to those observed elsewhere in North America and in Europe. Furthermore, our study documented the first known case of invading brown trout establishing resident populations, though the majority of individuals remain anadromous. The establishment of brown trout in the Renews River watershed has the potential to impact native populations of Atlantic salmon and brook charr. Furthermore, brown trout continue to spread, invading and establishing populations in other Newfoundland watersheds. Although brown trout invasion was occurring at a modest 4km/yr (Westley and Fleming 2011), and that minimal mixing between watersheds was occurring, at least from other systems into the Renews River watershed (Veinott et al. 2012), the finding that local species distribution patterns mirror those observed elsewhere after approximately 125 yrs is alarming. COSEWIC has recently recommended that southern Newfoundland populations of Atlantic salmon be listed as threatened (COSEWIC 2010), and impacts to brook charr, through the observed species distribution patters, are already occurring. Since brown trout are an introduced species in eastern Canada, and because they continue to disperse to new watersheds and establish new populations, understanding what contributes to their invasion success and how they impact native fishes is fundamental to the sustainability of those native populations.

Distinguishing anadromous from freshwater resident fish

Our results confirm that the Sr88 concentration at the otolith core was not a function of the environment the individuals were collected from (Elsdon & Gillanders 2005) but rather the environment the mother was in while the eggs ripened (Kalish 1990). As well, it suggests that LA-ICP-MS can clearly distinguish between freshwater and marine environments using this "drilling" or retrospective technique. Since local water chemistry does not affect the otolith core chemistry, it's assumed that any difference in Sr88 concentration will be a function of the Sr88 content of the individual's yolk sack, which is influenced by the environment where vitellogenesis took place (Kalish 1990; Rieman et al. 1994). Secor and Rooker (2000) conducted a meta-analysis of the relationship between otolith Sr:Ca ratios and ambient salinity for 27 species and found a positive relationship, indicating that the use of otolith Sr concentration and Sr:Ca ratios can be an appropriate tool for describing migration across significant salinity gradients (Zimmerman 2005). It has been suggested that when using retrospective analyses of YOY otoliths, it would be unlikely to confine chemical sampling to just the otolith core (Chittaro et al. 2006). However, our results reveal that this technique is effective for the purposes of identifying YOY offspring of anadromous and resident fish.

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Tables

Table 2-1 **Summary of size metrics of YOY salmonids collected from the Renews River watershed; post emergence (June) and end of growing season (September) 2009). The ratios of species collected are conservatively biased. Additional sampling effort was carried out to collect between 30 – 60 individuals from each species in each river section; however, if enough fish from a species were collected, no additional individuals were recorded (even if they were captured).**

River Section	Species	Post Emergence					End of Growing Season				
		Number of Individuals	Average FL_U (mm)	SD (±)	Average DW_U (grams)	SD (±)	Number of Individuals	Average FL_U (mm)	SD (±)	Average DW_U (grams)	SD (±)
US	Atlantic Salmon	67	32.1	2.39	0.0557	0.0163	63	44.9	4.52	0.2003	0.0666
	Brook Charr	49	33.9	4.37	0.0641	0.0324	48	48.4	6.92	0.2238	0.1032
	Brown Trout	1	38.1		0.0947		17	46.3	3.83	0.1959	0.0517
MS	Atlantic Salmon	55	32.2	2.42	0.0534	0.0175	54	49.3	7.58	0.2899	0.1410
	Brook Charr	37	34.4	4.89	0.0701	0.0412	12	51.3	6.72	0.2682	0.1288
	Brown Trout	35	33.5	4.00	0.0710	0.0292	58	51.7	5.92	0.2835	0.1009
DS	Atlantic Salmon	33	36.1	3.75	0.0875	0.0307	53	53.9	5.05	0.3565	0.1050
	Brook Charr	3	37.8	2.95	0.0910	0.0272	4	52.5	3.99	0.3024	0.0726
	Brown Trout	58	34.6	3.39	0.0740	0.0264	58	52.4	3.89	0.2886	0.0781

Table 2-2 Summary of results from ANOVA comparing the Sr concentration within Renews River YOY otolith cores among species and river section. Analysis was completed for a random selection of 30 individuals from each species within each river section and sampling event; if less than 30 individuals were captured, all individuals were included in the analysis.

River Section	Species	Post Emergence				End of Growing Season			
		F-Statistic	P-Value	Anadromous	Resident	F-Statistic	P-Value	Anadromous	Resident
US	Atlantic salmon	$F_{[30,1364]}$ = 119.26	p < 0.001	26	4	$F_{[30,1364]}$ = 103.02	p < 0.001	29	1
	Brook Charr	$F_{[30,1364]}$ = 110.89	p < 0.001	5	25	$F_{[30,1364]}$ = 124.25	p < 0.001	10	20
	Brown Trout	$F_{[1,88]}$ = 668.27	p < 0.001	1	0	$F_{[17,792]}$ = 648.81	p < 0.001	16	1
MS	Atlantic salmon	$F_{[30,1364]}$ = 99.37	p < 0.001	30	0	$F_{[29,1320]}$ = 56.91	p < 0.001	30	0
	Brook Charr	$F_{[30,1364]}$ = 294.46	p < 0.001	6	24	$F_{[12,572]}$ = 77.67	p < 0.001	0	12
	Brown Trout	$F_{[30,1364]}$ = 76.10	p < 0.001	29	1	$F_{[30,1364]}$ = 170.98	p < 0.001	30	0
DS	Atlantic salmon	$F_{[23,1056]}$ = 42.10	p < 0.001	23	0	$F_{[30,1364]}$ = 50.55	p < 0.001	30	0
	Brook Charr	$F_{[3,176]}$ = 114.43	p < 0.001	0	3	$F_{[4,220]}$ = 326.89	p < 0.001	2	2
	Brown Trout	$F_{[30,1364]}$ = 108.32	p < 0.001	30	0	$F_{[30,1364]}$ = 141.26	p < 0.001	30	0

Figures

Figure 2-1 Study area; location of the Renews River watershed near Renews, Newfoundland. Numbers indicate sample sites.

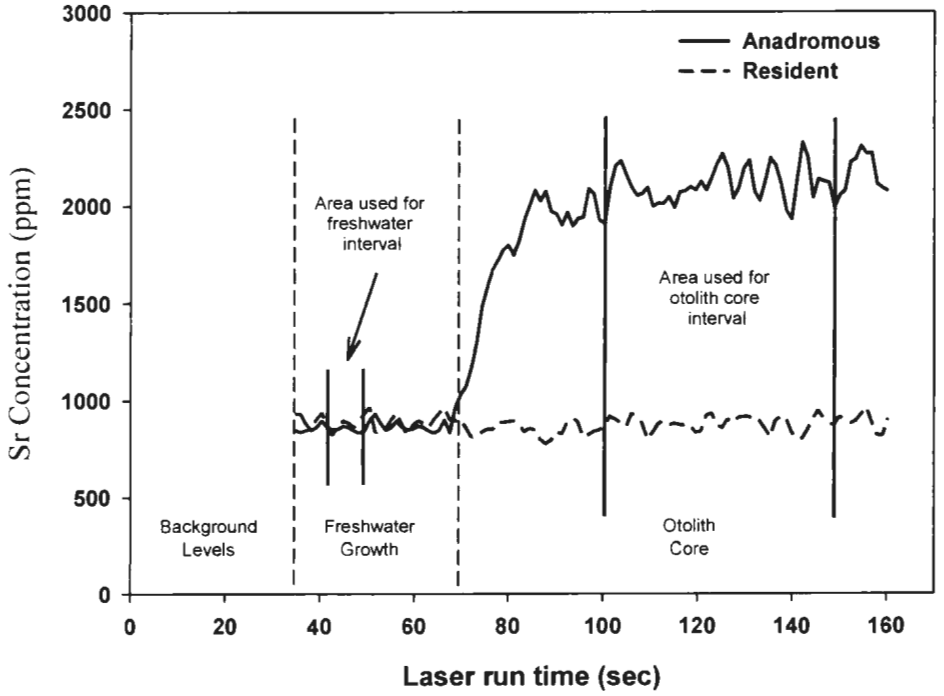

Figure 2-2 **Relationship between Sr concentration (ppm) and laser run time (sec) for an offspring of an anadromous (solid line) and resident (dashed line) brown trout captured at end of growing season (September) 2009. Dashed vertical lines delineate areas of background levels, freshwater growth and otolith core areas. Solid vertical lines delineate areas of the otolith used in the analysis.**

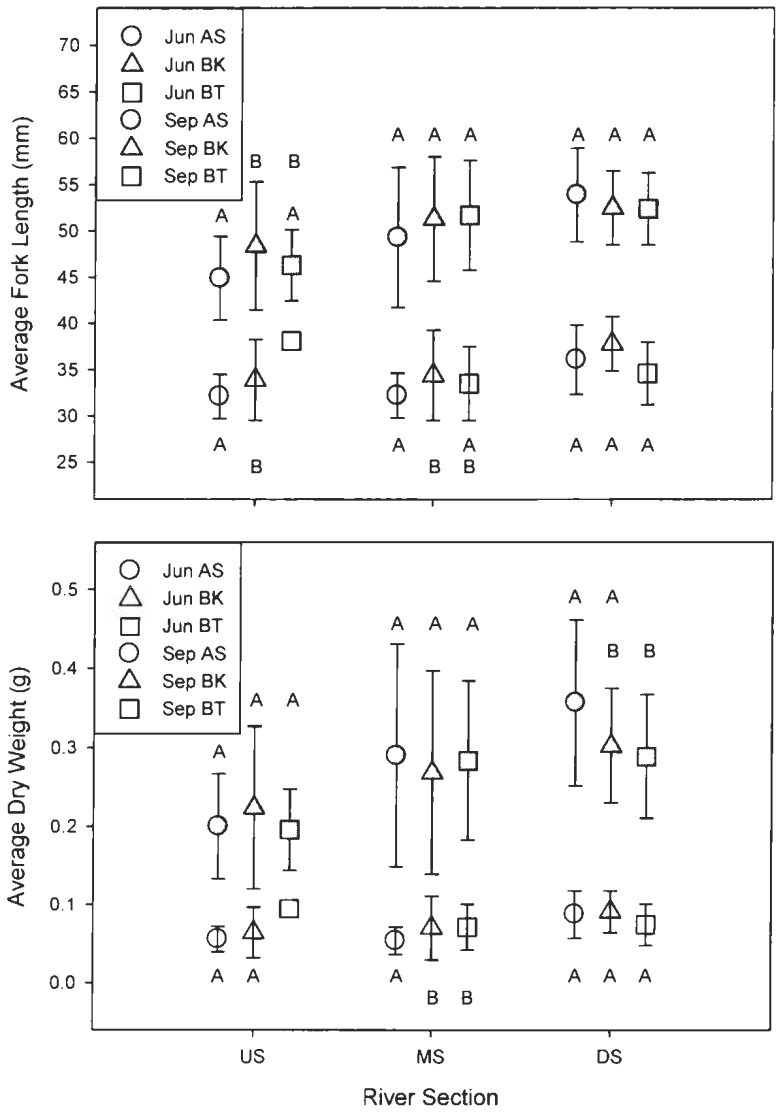

Figure 2-3 **Average FL_U and DW_U of YOY salmonids collected from the Renews River watershed at post emergence (June) and end of growing season (September) 2009. Atlantic salmon=AS, brook charr=BK, brown trout=BT; post emergence=Jun, end of growing season=Sep. Letters (A, B) represent Tukey grouping results for within river location analyses.**

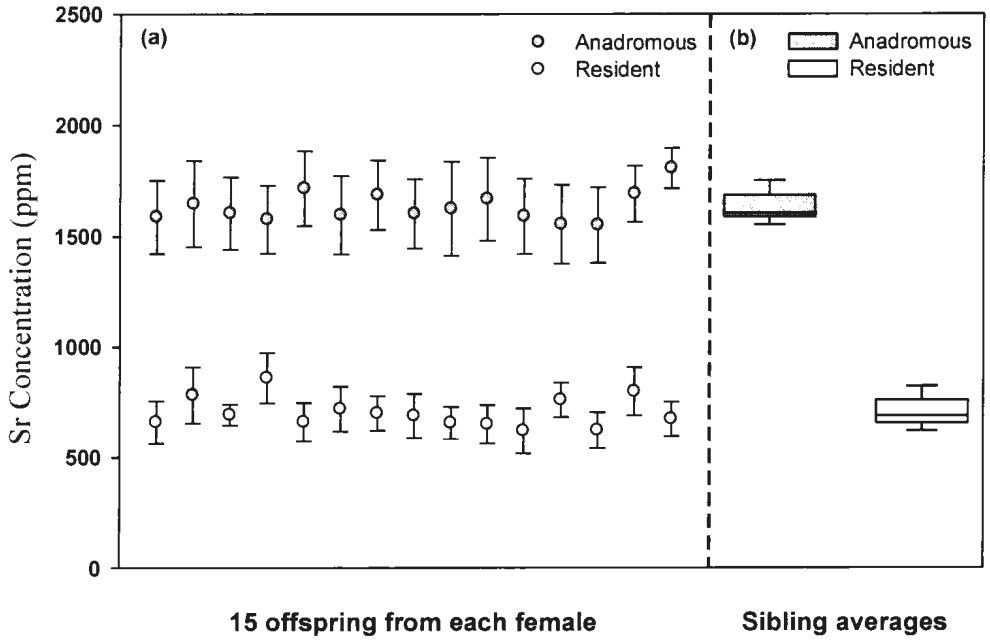

Figure 2-4 **Sr concentration (ppm) of otolith cores of 15 YOY brown trout siblings from an anadromous and resident female. Plot (a) data points represent the average Sr concentration (ppm) per offspring and error bars represent standard deviation between 54 values (Sr concentration readings) , and plot (b) box plots represent the average of the averages and shows the 5th/95th percentile (among fish).**

Appendix A-2

Table A2-1 Summary of YOY salmonids collected from 16 sample sites along the Renews River watershed at post emergence (June) and end of growing season (September) 2009. The ratios of species collected are conservatively biased. Additional sampling effort was carried out to collect between 30 – 60 individuals from each species in each river section; however, if enough fish from a species were collected, no additional individuals were recorded (even if they were captured).

River Section	Sample Site	Species	Catch June	Catch September	Sr:Ca *
US	16	Atlantic Salmon	32	11	
		Brook Trout	17	13	0.00543
		Brown Trout	0	0	
	15	Atlantic Salmon	0	0	
		Brook Trout	20	22	0.00465
		Brown Trout	0	0	
	14	Atlantic Salmon	0	0	
		Brook Trout	7	0	N/S
		Brown Trout	0	0	
	13	Atlantic Salmon	10	13	
		Brook Trout	5	12	N/S
		Brown Trout	0	6	
	12	Atlantic Salmon	10	29	
		Brook Trout	0	1	N/S
		Brown Trout	1	11	
	11	Atlantic Salmon	15	10	
		Brook Trout	0	0	N/S
		Brown Trout	0	0	
	10	Atlantic Salmon	1	0	
		Brook Trout	1	0	0.00426
		Brown Trout	0	0	
	9	Atlantic Salmon	36	30	
		Brook Trout	16	6	0.00429
		Brown Trout	20	12	
MS	8	Atlantic Salmon	0	0	
		Brook Trout	7	2	N/S
		Brown Trout	9	0	
	7	Atlantic Salmon	9	5	
		Brook Trout	5	0	N/S
		Brown Trout	3	24	
	6	Atlantic Salmon	9	19	
		Brook Trout	8	4	0.00438
		Brown Trout	3	22	
	5	Atlantic Salmon	0	4	
		Brook Trout	0	1	0.00574
		Brown Trout	10	10	
	4	Atlantic Salmon	8	43	
		Brook Trout	0	1	0.00437
		Brown Trout	8	11	
DS	3	Atlantic Salmon	5	3	
		Brook Trout	1	0	N/S
		Brown Trout	3	13	
	2	Atlantic Salmon	10	1	
		Brook Trout	1	0	N/S
		Brown Trout	20	7	
	1	Atlantic Salmon	10	2	
		Brook Trout	1	2	0.00406
		Brown Trout	17	17	

** Water samples were collected at eight of the 16 fish sampling sites; N/S indicates no sampling.*

Chapter 3: Season and life stage influence contrasting patterns of estuary use by native Atlantic salmon and invasive brown trout

Abstract

Brown trout were introduced to Newfoundland in the late 1800s and are now established in watersheds on the Avalon, Burin and Bonavista peninsulas, where native populations of salmonids already exist. Many of these systems include estuaries, which are extensively used by brook charr and juvenile salmon; yet, knowledge of seasonal estuary use by brown trout outside of their native range is limited. We investigated the seasonal use of estuary habitat by native Atlantic salmon and brook charr and introduced brown trout during parr, smolt and adult life cycle stages in eastern Newfoundland. Abundance of adult brown trout was relatively consistent throughout the year, but capture of adult Atlantic salmon was limited to August (28:1 brown trout to salmon caught). Abundance of parr and smolt was greater in spring and summer, a pattern consistent between species, with the exception of a large pulse of salmon smolt during one day. Excluding this pulse, brown trout were more abundant throughout the study (ratio of 2.4:1). The constant presence of invasive brown trout raises concerns for native salmonids, particularly Atlantic salmon on Newfoundland's south coast, which have been recently recommended for listing as threatened by the Committee on the Status of Endangered Wildlife in Canada.

Introduction

Migration is defined as a return movement between geographically separated areas consisting of different habitats (McKeown 1984) and is generally favoured when the fitness of individuals using multiple habitats exceeds the fitness of individuals using a single habitat (Gross 1987). In temperate latitudes, the seas are generally more productive than fresh waters and some groups of freshwater fish regularly migrate to salt water (i.e., become anadromous); these migrations are often facultative (e.g., many anadromous species do not have to go to sea to complete their lifecycles). Anadromous fish have an assumed adaptive advantage, where larger body size yields increased fecundity in females and a competitive advantage during spawning in males (Tallman et al. 1996; Morita and Takashima 1998). Furthermore, offspring of anadromous fish are afforded potential advantages during early stages of development through the transfer of energy resources acquired at sea (Jardine et al. 2008). However, anadromous individuals are believed to have higher mortality rates than fish that remain in fresh water through increased natural predation and human fishing pressure where anglers target anadromous individuals because of their larger body size (Roche 1992; Dieperink et al. 2002; Koed et al. 2006).

In many watersheds, anadromous migration includes movement through estuary habitat. Estuaries are semi enclosed coastal water bodies, where abrupt temporal changes in salinity, temperature, oxygen and turbidity occur due to the influence of tides and mixing of marine and fresh waters (Methven et al. 2001). These characteristics make estuaries important transitional zones, as well as highly productive rearing and feeding habitats, for both marine and freshwater fish species (Etheridge et al. 2008). Estuaries, therefore, offer potential advantages to migrating individuals; however, how facultative anadromous species use different habitats when introduced to novel areas is poorly understood.

Brown trout (*Salmo trutta*), which is native to Eurasia, is one of the most commonly transplanted salmonid species and has been successfully introduced nearly world-wide (Klemetsen et al. 2003). Following their introduction to various systems, resulting competition with native species has become of great concern in many regions, including New Zealand (McDowall 2006), South America (Pascual 2007), and North America (van Zyll de Jong et al. 2004). As a result of their impacts to native fishes, brown trout is listed as one of the '100 worst invasive alien species' by the Invasive Species Specialist Group (Lowe et al. 2000).

In North America, competition between introduced brown trout and native populations of Atlantic salmon (*Salmo salar*) (Fenderson 1954) and brook charr (*Salvelinus fontinalis*) (Waters 1983) in rivers has long been a concern. One of the earliest introduction sites of brown trout in North America is the island of Newfoundland; near the city of St. John's in the late 1800s (Scott and Crossman 1964; Hustins 2007). Introduced individuals were initially descendants of non-migratory females; however,

brown trout are spreading and are now established in numerous watersheds on the Avalon, Burin and Bonavista peninsulas (Westley and Fleming 2011). It has been suggested that these new populations were established by straying anadromous individuals (van Zyll de Jong et al. 2004; Westley and Fleming 2011), which is a pattern documented in other brown trout invasions (Launey et al. 2010). However, ironically anadromy is often implicated in salmonid introductions that fail to establish self-sustaining populations outside of their native ranges; straying anadromous fish continue to stray rather than return to the same stream in successive years (Quinn 2005).

Salmon stocks in eastern Canada have been in decline since the 1970s and have yet to recover (DFO 2006). Meanwhile, brown trout continue to disperse to adjacent watersheds, many of which include estuary habitat, and establish new populations in Newfoundland (Westley and Fleming 2011). Atlantic salmon are native to both western Europe (where they co-evolved with brown trout) and eastern North America (where brown trout are not native), and brook charr are native to eastern North America (they did not co-evolve with brown trout). In Europe, brown trout (adults and juveniles) use estuaries extensively (Knutsen et al. 2001; Knutsen et al. 2004; Rikardsen et al. 2006), whereas Atlantic salmon generally do not (Klemetsen et al. 2003; Jonsson and Jonsson 2009). However, juvenile salmon have been repeatedly shown to exploit estuaries in eastern Canada (Cunjak 1992; Thorpe 1994); this may or may not be related to the absence of brown trout, which are a known predator to juvenile salmon (Larsson 1985). Interestingly, our review yielded only one study, conducted in Denmark, which investigated estuary habitat use by both species within the same watershed (Koed et al. 2006). Little is known of brown trout estuary use outside of their native range (Scott and Crossman 1964; Hustins 2007). Charr, in general, are less tolerant of seawater than Salmo (Rounsefell 1958; McCormick et al. 1985). Anadromous brook charr may spend only a few weeks (Curry et al. 2010) out of freshwater where they remain in estuary habitat, rarely being found far from river mouths and in full strength seawater (Dutil and Power 1980; Curry et al. 2006; Curry et al. 2010). Therefore, there is great but undocumented potential for overlap between brown trout, brook charr and Atlantic salmon estuary use in eastern North America.

Clearly, our knowledge of estuary habitat use by salmonids, and of what relative advantages there are to using estuary habitat, is still limited. Furthermore, there is a dearth of information comparing estuary habitat use by individuals at different life cycle stages (i.e., parr, smolt and adult) and how this is a function of co-occurring species and season. Since Atlantic salmon, brown trout, and brook charr are known to use estuary habitat, understanding how individuals at different life cycle stages potentially interact is of great importance. Thus, we hypothesized that estuary use within a life cycle stage varies (i) seasonally, and (ii) among species. Results from this study could have implications concerning the management of these three salmonids, as well as aid in the recovery of native Atlantic salmon stocks.

Methods

Study area

Sampling was conducted in the Renews Estuary (46o55'N, 52o56'W), Newfoundland, Canada, approximately 90 km south of St. John's (Figure 3-1). The estuary is connected to a narrow bay, approximately 0.5 km by 3 km, before becoming open-ocean. The estuary is relatively small and shallow (18.6 hectares) with a tidal amplitude of 0.7 – 1.5 m. Many shallow areas become dry during peak low tides; however, deeper trenches remain filled with water at all times. The estuary is largely enclosed by a man made break water that also protects it from easterly winds and wave action. The substrate consists of a gently sloping bottom of small rocks and gravel, which extends 5 – 10 m offshore. Silt and mud accumulates with increasing distance from shore and aquatic vegetation, including eel grass (*Zostera marina*) and Rockweed (*Ascophyllum nodosum*), is abundant throughout. Harbour seals (*Phoca vitulina*) are regularly present, and a small island supports a seasonal common tern (*Sterna paradisaea*) nesting colony (Veinott 2009). Anglers regularly target anadromous brown trout in the estuary. Atlantic salmon reproduce throughout the watershed (as evidenced by presence of young of year), brown trout in the lower main river, and large numbers of brook charr in the upper tributaries (Warner 2013).

Fish Sampling

Preliminary exploration, testing, and careful planning was undertaken to ensure unbiased seasonal comparisons could be made. Standardized sampling of the estuary was conducted over an entire year, commencing in May 2009. Sampling was bi-monthly, and timed to occur when peak high tide was between the daylight hours of 10:00 – 16:00. Two days within each high tide cycle were sampled and all sampling occurred within a four hour time window (two hours ± peak high tide) in order to ensure consistency. Temporary ice cover made sampling impossible in one tide cycle in each of December 2009, January 2010, and February 2010; however, the other tide cycle within each of these three months was successfully sampled.

Seven sampling sites were specifically chosen because they could be consistently sampled throughout the duration of this study. Two gear types (beach seine and gill net) were used because they could effectively sample the estuary and capture fish at different life cycle stages (i.e., parr, smolt and adult). The goal was to examine temporal changes in the combined catch from all standard daily fishing effort. Five sites were sampled using sinking gill nets. These five sites were chosen to sample habitats that could not be seined. Each gill net had two-panels, 2.54 and 5.08 cm stretched mesh, that measured 30 m by 1.8 m. Larger mesh was not used in order to increase effort on sizes of fish that would likely be captured with reasonable frequency. The exact location and fishing time of each gill net set was standardized. Gillnets were set for one hour in order to avoid sampling mortality, as all fish were released. Each gill net site was sampled once each day, except one site, which was sampled twice each day.

Two different sites were sampled using a large beach seine that was deployed from a boat. The locations were chosen because they were free of large rocks, which would have obstructed the movement of the seine (no other suitable sites existed). The net measured 22 m by 2 m, with 35 m ropes attached to spreader bars on the end of each wing. The wings of the seine consisted of 19mm stretched mesh, and the codend consisted of 13mm stretched mesh, with a 9mm liner. Standardized landmarks were placed on shore above the peak high tide water level. The seine was deployed 35m from shore between these landmarks and then pulled onshore (1 pull = 1 sample). The area sampled during each pull was approximately 550 m2. Each seine site was sampled twice (2 pulls) each day. All fish captured, using both sampling methods described above, were counted, identified to species, and fork-length was recorded.

Identifying the life cycle stage of an individual can be difficult because the expression of phenotypic traits, including color and body shape, can vary between populations of salmonids. Because clear distinction between parr, smolt and adult life cycle stages of Atlantic salmon and brown trout could not be consistently achieved (brook charr were not captured – see below), fish were grouped into 3 size classes (Figure 3-2). Individuals that measured $\leq$ 100mm were classified as small, and were typically parr. Fish that measured > 100mm and < 250mm were classified as medium, and typically represented individuals that are undergoing or have completed smoltification (typically called smolts), which is the physiological transition for life in the marine environment. Individuals that measured $\geq$ 250mm were classified as large, most of which would have been adults.

Passive Integrated Transponder (PIT) tags were implanted in most salmonids measuring $\geq$ 45 mm using 8mm, 12mm and 23mm tags, depending on the size of the individual fish. A total of 285 tags were deployed and included 97 Atlantic salmon and 188 brown trout. However, because of the low number of recaptured individuals (only 2 fish that were tagged were recaptured), these data are not presented.

Temperature Data

Four temperature loggers were deployed in the study area; two in the estuary and two in the harbour. Each logger recorded temperature every hour and was installed next to a large boulder and always remained under water (~ 2m) and free of ice cover. Temperature data retrieved from each logger was averaged to month, and the average of the two loggers was chosen to represent the monthly water temperature of the estuary and harbour. Temperature data was also recovered from Department of Fisheries and Oceans Canada (DFO) Station 27 (47°32'N, 52°35'W; located approximately 8 km outside of St. John's Harbour) from May 2009 to April 2010. Station 27 is the first hydrographic monitoring station in the DFO standard St. John's to Flemish Cap transect, which was established in 1946. The station is located within the Avalon Channel and is used to represent typical water temperature on the continental shelf. The average monthly surface temperature (< 10 meters depth) was selected to represent water temperature of the open ocean, except for January and February 2010 when data were not collected.

Statistical analyses

Statistics were conducted using the R statistical package (i.e., base package) version 2.10.0. Significance was set at $\alpha = 0.05$ and assumptions of parametric statistics were checked by examining model residuals.

Fish catch precision

To determine the variability at each sampling level, variance components for the number of fish captured using each sampling gear (seine and gill nets) were examined for random factors with a fully nested analysis of variance (ANOVA). Response variables were catch numbers of large, medium and small size classes for Atlantic salmon, brown trout, and a pool of all other species. Random factors for the seine net catches were season, month, tide cycle, day, site and set, while gill net catches were season, month, tide cycle, day and site (the second set was removed from the one duplicated gill net site for the purposes of this analysis). Large Atlantic salmon were not captured using seine nets and small Atlantic salmon and brown trout were not captured using gill nets. In response to the high incidence of zero catch, data were transformed using $Catch = \sqrt{(Catch + 0.5)}$. This produced the model of best fit and is a recommended transformation when working with data with a high incidence of zeros (Sokal and Rohlf 1995; Zar 1998).

Fine scale variability was much more pronounced than large temporal trends. Results from the variance component analysis (Figure 3-3) show that the majority of the variability in catch is among sets and sites, when sampling with seine nets (74% average across fish species and size classes), and among sites, when sampling with gill nets (92% average across fish species and size classes). These results illustrate that catch results can vary greatly among samples and sites and demonstrate the importance of repeated sampling.

Seasonal trends in Salmonid Catch Data

To evaluate larger (i.e., seasonal) trends, catch results for each size class of Atlantic salmon and brown trout, using both seine nets and gill nets, were pooled by species among sampling events and summed to each tide cycle. Tide cycle was the lowest sampling level that was determined to be an independent sampling unit. Summing to tide cycle also minimizes potential influences from high catch variability from lower sampling levels as determined from the variance component analysis (ie. sets and sites). As above, catch results data were transformed using $Catch = \sqrt{(Catch + 0.5)}$.

Catch results for Atlantic salmon and brown trout were analyzed using ANOVA. Although brook charr are present in high numbers in Renews River (Warner 2013), due to low catches in the estuary (see results) they are not considered further here. The dependent variable was *number of individuals* (catch) and the independent variables were (i) *size class*, (ii) *species*, (iii) *season* and (iv) *month(season)*, and all appropriate interaction terms. Ice out in Newfoundland typically occurs in late April and we thus offset calendar seasons by one month (spring = May-June-July). In no cases were

interaction terms found to be significant; therefore, they were removed and the simplified model was run and those results are reported.

Results

A total of 19 848 fish representing 16 species were captured during the 12 month sampling period (Table 3-1). Threespine stickleback (*Gasterosteus aculeatus*) (62%), fourspine stickleback (*Apeltes quadracus*) (26%), and sculpin sp. (*Cottidae*) (5%) accounted for 93% of the total catch. These species of small fishes were also the most consistently captured. Seasonal trends (i.e., catch results) of all fish species are summarized in Appendix 3-A, including month (Table 3-A1), tide cycle (Table 3-A2), and Julian day (Table 3-A3).

A total of 1024 salmonids representing three species were captured; Atlantic salmon ($n = 770$), brown trout ($n = 250$) and brook charr ($n = 4$). Salmonids accounted for 5% of the total number of fish collected. Atlantic salmon accounted for 75% of salmonids collected (most of which were caught on 1 day, see below) and brown trout 24%, although brown trout were the dominant large fish captured.

Estuary use varied by season, species and size class. Figure 3-4 summarizes the average monthly water temperatures and catch of (a) small, (b) medium and (c) large Atlantic salmon and brown trout from May 2009 to April 2010. Data points represent average catch per month and error bars represent standard deviation between two high-tide cycles. Because of ice cover during one high-tide cycle in December 2009, as well as January and February 2010, each datum for these months represents the total catch for the one high-tide cycle sampled, and has no error bars. Significant results are reported for size ($F_{[2,131]} = 6.90$, $p = 0.001$) and season ($F_{[3,131]} = 4.81$, $p = 0.031$). In order to analyze seasonal estuary use within each size class by these two salmonid species, three separate ANOVA's were run, one for each size class (small, medium and large).

Small salmonids (typically parr) were captured from May – August 2009, and again in April 2010 (none from September 2009 – March 2010), and were most abundant during spring. The number of individuals differed significantly among seasons ($F_{[3,43]} = 107.66$, $p < 0.001$), but not among months within a season ($F_{[8,43]} = 0.03$, $p > 0.999$) or species ($F_{[1,43]} = 0.07$, $p = 0.791$).

Medium sized fish (typically smolts) were the most frequently captured size class and were present in every month except January and March. Overall, total catches did not differ significantly among seasons ($F_{[3,43]} = 2.74$, $p = 0.109$), months within a season ($F_{[8,43]} = 1.17$, $p = 0.348$) or species ($F_{[1,43]} = 0.00$, $p = 0.982$). A single large pulse of medium fish was captured on May 13, 2009 and consisted of 673 Atlantic salmon and 54 brown trout (accounted for 92% and 27%, respectively, of medium Atlantic salmon and brown trout captured throughout the study). This aggregation of salmonids was attributed to the annual smolt run (or seaward migration) and was absent the subsequent day; it greatly affects relative abundance comparisons between Atlantic salmon and brown trout

on an annual basis (see discussion). Because it was an outlier when compared to the rest of the year, this data point was removed and the model was re-run. When we did this, the number of individuals did not differ significantly among seasons ($F_{[3,43]} = 3.81$, $p = 0.057$); but there were significantly more brown trout than Atlantic salmon ($F_{[1,43]} = 16.32$, $p < 0.001$), and catches differed significantly among months with a season ($F_{[8,43]} = 3.24$, $p = 0.009$).

Large fish were captured from May – October, December 2009, as well as April 2010, with large brown trout significantly more numerous (28:1) than large Atlantic salmon ($F_{[1,43]} = 6.08$, $p = 0.019$). Only one large Atlantic salmon was captured during this study; this individual was captured on August 13, 2009 with a fork-length of 562 mm. Large brown trout catches were steady, with the exception of a single pulse on May 13, 2009 .This pulse was attributed to the annual seaward migration of overwintering adults. Overall, the number of individuals did not differ significantly among our definition of seasons ($F_{[3,43]} = 1.82$, $p = 0.215$), or months within a season ($F_{[8,43]} = 0.72$, $p = 0.674$), although it is evident that catches were consistently highest in April-June (Figure 3-4, spring was considered May-July).

Average water temperature in the Renews estuary was consistently higher than in the harbour and the ocean from May – November 2009, and reached a maximum in August 2009 of 16 ^{o}C (Figure 3-4). Based on the available temperature data from our loggers and from DFO Station 27, the average water temperature in the Renews estuary, harbour, and the ocean remained above 0^{o}C throughout the winter months. Water temperatures measured at DFO Station 27 during the winter of 2010/11 (December 2010, March and April 2011) were the warmest on record, since 1946 (Fitzpatrick 2011).

Discussion

This study represents the first detailed investigation of seasonal estuary use by Atlantic salmon and brown trout, outside of the native range (i.e., Europe) of the latter, and our results suggest that estuary habitat use is an interactive function of species, life stage, and time of year. Our findings suggest that invasive brown trout in eastern Newfoundland behave similarly to native brown trout from Europe; both use estuary habitat throughout the year. Our results also showed that juvenile Atlantic salmon use estuary habitat throughout the spring and summer and that many are of appropriate prey size for adult brown trout; which have been shown to be a key predator to juvenile salmon during seaward migrations through estuary and coastal habitats in Europe (Larsson 1985; Koed et al. 2006). These findings present a potentially serious threat to the recovery and sustainability of native Atlantic salmon populations in parts of eastern North America. There is a lack of anadromous brook charr in this watershed, which may or may not be related to the presence of invasive brown trout.

Atlantic salmon and brown trout are closely related with some degree of overlapping niche ranges; they exhibit both anadromous and resident migration strategies, and are known to utilize estuary habitat to some degree (Scott and Crossman 1964;

Klemetsen et al. 2003; Jonsson and Jonsson 2009). Though we found that parr and smolt abundance was greater in spring and summer than in fall and winter, a pattern that was generally consistent between species, pronounced differences were present between species in large individuals. Abundance of adult brown trout was considerably higher than salmon (28:1) and was relatively consistent throughout the year. Only one adult Atlantic salmon was captured during the study period; however, two additional adult salmon, kelts (post-spawned adults returning to sea), were caught during preliminary exploratory sampling in April 2009. These individuals were captured at different sites than those selected for the standardized sampling methodology for this study. As such, these individuals were not included in analyses.

Estuary habitat use by adult brown trout has been well documented in Europe, with studies revealing they utilize estuaries for feeding and rearing from spring through fall months (Knutsen et al. 2001; Knutsen et al. 2004; Rikardsen et al. 2006). Whereas most populations of anadromous Atlantic salmon undergo long distance migrations, brown trout tend to remain in coastal areas, including estuaries, and do not migrate very far from their home rivers (O'Connell 1982; Klemetsen et al. 2003; Jonsson and Jonsson 2006). Our results support these studies as adult brown trout were consistently captured throughout most of the year, and suggest that, like in European systems, estuaries are important feeding habitats for adult brown trout. The value of estuary habitat is further emphasized by recent work by Westley and Fleming (2011) that found that the majority of systems in Newfoundland colonized by introduced brown trout include estuary habitat. Brown trout adults have also been observed in estuaries during winter months and it has been suggested that they use estuary habitat as a refuge from low-temperature ($< 2^{o}C$) winter conditions in marine waters, as hypo-osmoregulatory capacity of these individuals becomes compromised (Thomsen et al. 2007; Jensen and Rikardsen 2008). Unexpectedly, we did not detect a peak in abundance of brown trout in winter in the Renews estuary, which may or may not be due to winter 2010 being the warmest on record (Fitzpatrick 2011).

Unlike brown trout, Atlantic salmon adults generally do not utilize estuary habitat, but simply move through estuaries during spring spawning runs or return movements from sea (Klemetsen et al. 2003; Jonsson and Jonsson 2009). However, they may hold in estuaries for several months if river water levels and flow are insufficient for passage upstream on return migrations (Saunders 1960; Soloman and Sambrook 2004; Jonsson et al. 2007; Jonsson and Jonsson 2009). Atlantic salmon kelts have also been observed in estuaries during winter months if suitable overwintering habitat in the river is limited (Hubley et al. 2008). This did not occur in the Renews estuary in 2009 and our results support the other studies in that estuaries are not important feeding habitat for adult Atlantic salmon.

Salmon and trout parr use the Renews estuary extensively. We captured parr from both species from May – August 2009 and again in April 2010, with the highest catches occurring in spring. This is consistent with observations from a few other studies and suggests that Atlantic salmon and brown trout parr use this estuary for feeding and rearing

during spring and summer months (Cunjak et al. 1989; Knutsen et al. 2001). However, like these other studies, our results suggest a paradox with the physiological development of individuals in the parr life cycle stage. Parr have low salinity tolerances and, as a result, are not yet physiologically adapted to life in fully marine waters (Parry 1960; Jonsson and Jonsson 1998). However, estuarine waters are dynamic, with spatial and temporal variability in salinity concentrations (Methven et al. 2001). Salinity levels in the Renews estuary (average surface and bottom levels were 3.2 ppt and 7.9 ppt, respectively) (Veinott 2009) were similar to those measured in Western Arm Brook estuary (Cunjak et al. 1989), were Atlantic salmon parr were captured, and on the Norwegian Skagerrak coast (Knutsen et al. 2004), where juvenile brown trout were captured. This unique characteristic of estuary habitat appears to allow parr to tolerate estuarine waters for short periods of time for feeding. This may also provide an ideal transitional habitat for parr to complete smoltification for their migration to sea. During winter, the hypo-osmoregulatory capacity of these individuals becomes compromised in brackish water (Thomsen et al. 2007) and may force individuals that cannot complete smoltification to return to freshwater. This may partially explain the absence of parr of either species during September 2009 through March 2010; though water temperature in the estuary was still relatively warm during September and October, 2009. Alternatively, because parr utilize estuary habitat for feeding during the summer, the absence of parr during the fall could be the result of individuals growing and becoming smolts, as smolts from both species were captured during the fall. Otherwise, predation may also explain the absence of parr during September 2009 through March 2010, as a variety of predators are present in the Renews Estuary, including birds, seals, and larger fish, and are known to feed on fish in the estuary (Veinott 2009).

Smolts from both species were consistently captured throughout most of the year and were more abundant than parr or adults. Though seasonal estuary habitat use by these two species was not significantly different, most of the individuals were caught on one day and were mostly Atlantic salmon (12.5:1 on May 13, 2009). Atlantic salmon smolts are known to pass through estuaries during seaward migrations, but some studies have shown them to be present in estuaries throughout spring and summer months (Cunjak et al. 1989; Klemetsen et al. 2003; Pinder et al. 2007). Since the majority of salmon smolts were captured on one day, our results suggest that the preponderance of salmon smolt pass through the Renews estuary during their seaward migration. Brown trout are more likely than Atlantic salmon to remain in estuaries for rearing and feeding (Knutsen et al. 2001; Klemetsen et al. 2003). Although a pulse of brown trout smolt was captured on one day, 73% of medium brown trout captured in this study were collected during the rest of the sampling events. Excluding the pulses caught on May 13, 2009, brown trout smolts were more abundant than salmon throughout the rest of the study, at a ratio of 2.4:1. These results suggest that estuaries continue to be important for brown trout smolt outside of their native range. Furthermore, these results emphasize how the smolt run greatly affects relative abundance comparisons between Atlantic salmon and brown trout on an annual basis. Finally, similar to discussions on parr described above, predation may also help explain the large drop in smolts as this has been documented in the Renews estuary (Veinott 2009) and in other studies (Koed et al. 2006).

Given that only four brook charr were captured during this study (during spring and summer), meaningful comparisons could not be investigated. However, brook charr are present in relatively high abundance in the Renews River and otolith microchemistry analysis of brook charr from this system concluded that the vast majority are offspring of resident fish (Warner 2013). Therefore, the absence of brook charr in the Renews Estuary may be the result of the low incidence of anadromous brook charr in this system. Curry et al. (2010) found that brook charr < 20 cm typically remain in fresh water, while laboratory studies suggest a minimum body size of 18 cm is required to accommodate the physiological changes needed for osmoregulation in full-strength salt water (Naiman et al. 1987; McCormick 1994). Our results, though limited, support these studies as three of four brook charr captured measured greater than 20 cm, with one measuring 15 cm. Because brown trout and brook charr use estuaries extensively, there is great potential for competition between these two species in North America. Whether the lack of anadromous brook charr in this system is related to the presence in invasive brown trout is unknown.

Interestingly, these brown trout have successfully invaded the Renews River watershed in spite of potential resistance. This watershed has established native populations of Atlantic salmon and brook charr, which may present biotic resistance to invading brown trout (Moyle & Light 1996). This watershed is also relatively pristine, with little known anthropogenic disturbance; a characteristic not normally associated with successful invasions (Fausch et al. 2009). Finally, the Renews River watershed was not originally stocked with brown trout, and stocking efforts in the vicinity of St John's (approx. 90 km away) ceased in the early 20th century (Hustins 2007); removing continuous pressure on native species and nullifying a numeric advantage. In spite of these potential resistance factors, brown trout have successfully invaded and are now present throughout much of the Renews River watershed.

How effective our sampling methods were at catching fish, particularly large Atlantic salmon, is not completely known. We did catch three large salmon and many large brown trout and, thus, conclude that during sampling days relative catches between species should be accurate. As we only sampled two days every two weeks, the peak run of returning adult salmon may have passed through the estuary between sampling events. When fish are caught they obviously must be in the area, but if they are not captured it doesn't necessarily mean they are not present. However, using standard sampling methods and a robust approach, our study should accurately represent major trends, but precision for small scale interpretation is poor. We have interpreted the absence of captured fish over multiple sampling events (sets, sites, days, and tide cycles) as meaning they are indeed absent from the estuary.

In summary, we document species differences in seasonal estuary use based on life stage. The Committee on the Status of Endangered Wildlife in Canada has recently recommended that hundreds of Atlantic salmon populations be listed as endangered; those from southern Newfoundland are considered threatened (COSEWIC 2010). Our

finding of nearly year-round estuary use by adult brown trout and seasonal (spring and summer) estuary use by juvenile salmon presents a potentially serious threat to the recovery and sustainability of eastern North America's native Atlantic salmon populations. With brown trout continuing to disperse to new systems, including those with threatened Atlantic salmon populations, more research is needed to better understand how they directly affect salmon. Additionally, although effects of invasive brown trout on brook charr in North American streams are well documented (Fausch and White 1981; Waters 1983; Korsu et al. 2007), how these processes transfer to estuaries is unknown.

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Tables

Table 3-1. Summary of fish collected in the Renews Estuary (May 2009 – April 2010)

Species Name	Common Name	Catch			
		Gill Net	Seine Net	Total Catch	Percent of Total Catch
Gasterosteus aculeatus	Threespine stickleback	3	12276	12279	61.865
Apeltes quadracus	Fourspine stickleback	1	5222	5223	26.315
Cottidae sp.	Sculpin sp.	2	959	961	4.842
Salmo salar	Atlantic salmon	6	764	770	3.879
Salmo trutta	Brown trout	28	222	250	1.260
Gadus ogac	Greenland cod	3	170	173	0.872
Gasterosteus wheatlandi	Blackspotted stickleback	0	96	96	0.484
Pseudopleuronectes americanus	Winter flounder	1	44	45	0.227
Anguilla rostrata	American eel	0	19	19	0.096
Osmerus mordax	Rainbow smelt	3	8	11	0.055
Clupea harengus	Atlantic Herring	3	4	7	0.035
Urophycis tenuis	White hake	0	6	6	0.030
Salvelinus fontinalis	Brook trout	0	4	4	0.020
Ammodytes americanus	American sandlance	0	2	2	0.010
Cyclopterus lumpus	lump fish	1	0	1	0.005
Pholis gunnellus	rock gunnel	0	1	1	0.005
Total				19848	100.000

Figures

Figure 3-1 Map showing location of the Renews Estuary, Newfoundland.

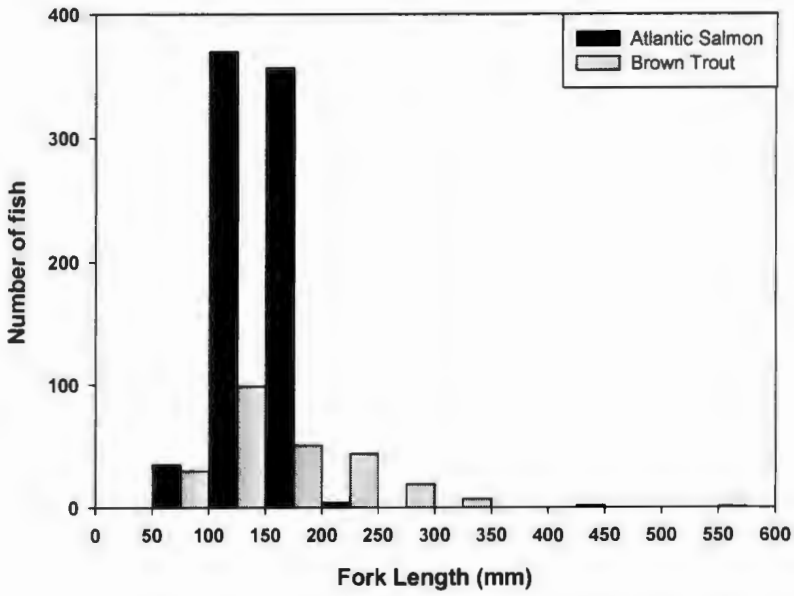

Figure 3-2 **Summary of the number of Atlantic salmon and brown trout captured by fork length. Individuals were grouped into 3 size classes: small (≤ 100 mm), medium (250 mm < 100 mm) and large (≥ 250 mm). Atlantic salmon (n = 770): 35 small, 734 medium and 1 large; brown trout (n = 250): 29 small, 193 medium and 28 large.**

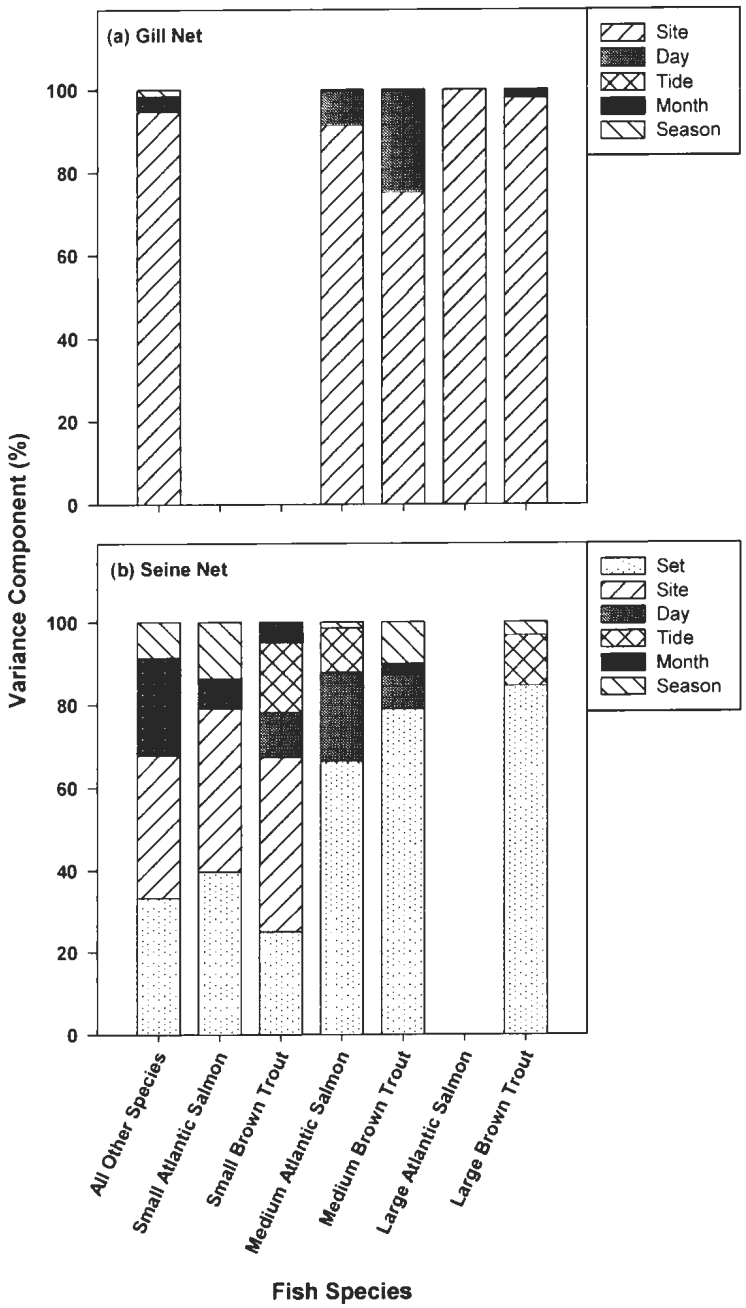

Figure 3-3 **Results of 11 full nested analyses of variance (ANOVA's) separating variance components for fish catch results (Catch #s) for (a) gill net sites and (b) seine net sites, among seasons (n=4), months within seasons (n=3), tide cycles within months (n=2), days within tide cycles (n=2), sites within days (n=2 for seines, 5 for gill nets) and, for seine net sites only, sets within sites (n=2). Fish species include Atlantic salmon, brown trout, and all other species.**

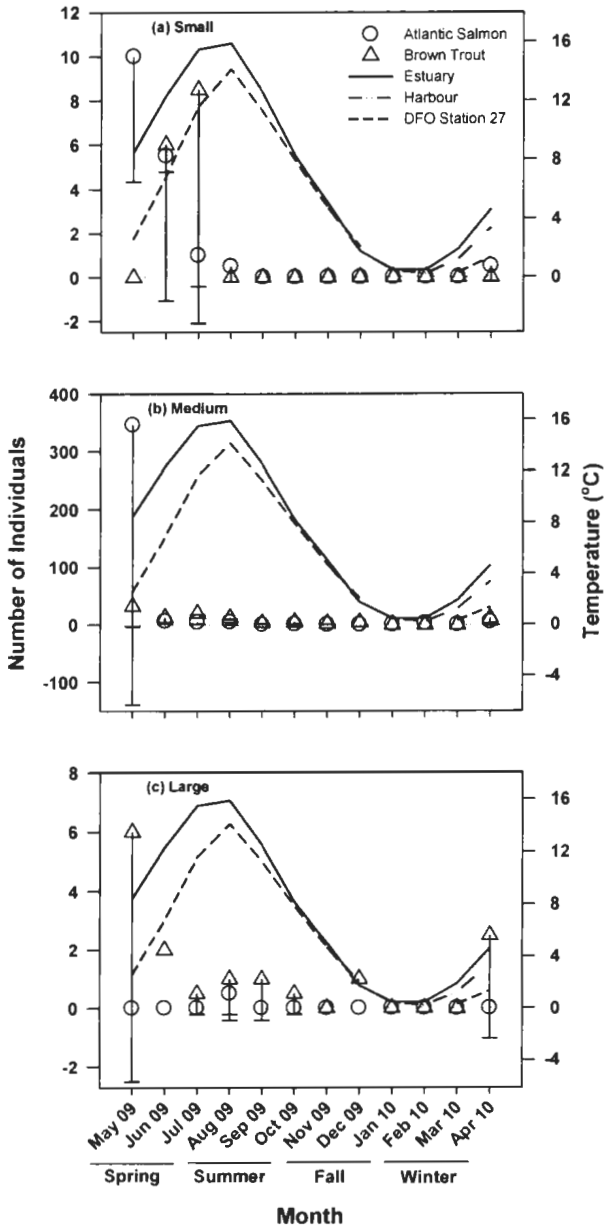

Figure 3-4 **Average monthly catch (± standard deviation) of (a) small, (b) medium and (c) large Atlantic salmon and brown trout and average monthly water temperature in the estuary, harbour and, DFO station 27. Number of Individuals was summed to tide cycle; data points represent average catch per month and error bars represent standard deviation between two high-tide cycles. Error bars are absent from December 2009 – February 2010 because only one tide cycle was sampled (due to ice-cover); all other data points where error bars are absent were because catch results from both tide cycles were identical. Temperature data was unavailable from the harbour for April-October 2009, and from DFO station 27 for January-February 2010. Months were grouped by seasons (spring = May, June, Jul; summer = Aug, Sep, Oct; fall=Nov, Dec, Jan; winter = Feb, Mar, Apr). Y-axis is at different scales for each size class.**

Appendix A-3

Table A3-1 Seasonal trends (catch by month) of all fish species captured during estuary sampling (May 2009 to April 2010).

	Month											
Species	May-09	Jun-09	Jul-09	Aug-09	Sep-09	Oct-09	Nov-09	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10
American Eel	0	2	12	5	0	0	0	0	0	0	0	0
Sculpin Sp	42	93	50	78	32	35	117	23	10	58	157	276
American Sandlance	0	0	0	0	0	2	0	0	0	0	0	0
Atlantic Herring	0	0	0	5	0	2	0	0	0	0	0	0
Atlantic Salmon	732	23	10	11	0	1	0	0	0	2	0	10
Brook Trout	2	0	1	0	0	0	0	0	0	0	0	1
Brown Trout	78	41	57	26	9	10	4	5	0	0	0	26
Black Spotted Stickleback	0	14	14	2	1	3	32	1	1	0	4	24
Fourspine Stickleback	265	585	1328	973	850	222	67	42	84	152	236	536
Greenland Cod	0	5	0	0	34	86	32	0	7	5	4	0
Lumpfish	0	1	0	0	0	0	0	0	0	0	0	0
Rock Gunnel	0	1	0	0	0	0	0	0	0	0	0	0
Rainbow Smelt	1	1	1	0	2	3	2	0	0	0	0	1
Threespine Stickleback	869	1337	1360	268	1701	2496	2045	80	97	55	331	2104
Winter Flounder	2	11	7	10	3	0	2	1	0	0	1	8
White Hake	0	0	0	6	0	0	0	0	0	0	0	0

Table A3-2 Seasonal trends (catch by tide cycle) of all fish species captured during estuary sampling (May 2009 to April 2010).

	Tide Cycle																				
Month	May-09		Jun-09		Jul-09		Aug-09		Sep-09		Oct-09		Nov-09	Dec-09	Jan-09	Feb-09		Mar-09		Apr-09	
Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
American Eel	0	0	0	2	0	12	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sculpin Sp	19	13	64	29	23	27	56	22	6	26	15	20	52	65	23	10	58	48	109	148	128
American Sandlance	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Atlantic Herring	0	0	0	0	0	0	2	3	0	0	1	1	0	0	0	0	0	0	0	0	0
Atlantic Salmon	704	9	10	13	4	6	10	1	0	0	1	0	0	0	0	0	2	0	0	7	3
Brook Trout	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Brown Trout	71	7	8	33	37	20	16	10	2	7	8	2	1	3	5	0	0	0	0	24	2
Black Spotted Stickleback	0	0	6	8	11	3	0	2	0	1	2	1	30	2	1	1	0	4	0	12	12
Fourspine Stickleback	94	111	288	297	539	789	519	454	404	446	109	113	34	33	42	84	152	152	84	370	166
Greenland Cod	0	0	5	0	0	0	0	0	2	32	31	55	20	12	0	7	5	3	1	0	0
Lumpfish	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rock Gunnel	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rainbow Smelt	0	1	1	0	0	1	0	0	0	2	2	1	2	0	0	0	0	0	0	1	0
Threespine Stickleback	143	201	675	662	787	573	223	45	530	1171	1529	967	1654	391	80	97	55	81	250	850	1254
Winter Flounder	0	2	5	6	1	6	6	4	2	1	0	0	0	2	1	0	0	1	0	2	6
White Hake	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A3-3 Seasonal trends (catch by Julian day) of all fish species captured during estuary sampling (May 2009 to April 2010).

	Julian Day																																										
Tide Cycle	May-09				Jun-09				Jul-09				Aug-09				Sep-09				Oct-09				Nov-09				Dec-09		Jan-10		Feb-10		Mar-10				Apr-10			
Species	133	134	147	148	162	163	177	178	193	194	208	209	224	225	237	238	252	253	265	271	281	283	295	297	313	314	328	329	357	358	1005	1006	1054	1055	1063	1068	1079	1080	1096	1097	1111	1112
American Eel	0	0	0	0	0	0	1	1	0	0	3	9	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sculpin Sp	9	10	12	1	18	46	19	10	14	9	11	16	24	32	3	19	3	3	12	14	6	9	14	6	25	27	27	38	8	15	6	4	17	41	14	34	70	39	74	74	66	62
American Sandlance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atlantic Herring	0	0	0	0	0	0	0	0	0	0	0	0	2	0	3	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atlantic Salmon	675	29	3	6	6	4	5	8	1	3	1	5	6	4	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	7	0	1	2
Brook Trout	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Brown Trout	64	7	2	5	3	5	16	17	32	5	11	9	5	11	6	4	0	2	4	3	4	4	2	0	1	0	2	1	5	0	0	0	0	0	0	0	0	0	22	2	0	2
Black Spotted Stickleback	0	0	0	0	2	4	5	3	5	6	1	2	0	0	0	2	0	0	0	1	0	2	0	1	4	26	1	1	0	1	1	0	0	0	4	0	0	0	9	3	7	5
Fourspine Stickleback	18	76	52	59	143	145	145	152	311	228	224	565	227	292	185	269	233	171	258	188	44	65	72	41	6	28	7	26	11	31	45	39	64	88	48	104	48	36	245	125	74	92
Greenland Cod	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0	1	1	13	19	11	20	28	27	8	12	7	5	0	0	4	3	2	3	1	2	1	0	0	0	0	0
Lumpfish	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rock Gunnel	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rainbow Smelt	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Threespine Stickleback	34	109	89	112	476	199	272	390	285	502	185	388	144	79	22	23	250	280	88	1083	1148	381	430	537	1084	570	115	276	13	67	65	32	26	29	62	19	14	236	704	146	1082	172
Winter Flounder	0	0	0	2	2	3	3	3	0	1	2	4	1	5	0	4	1	1	0	1	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	0	0	0	2	2	4
White Hake	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Chapter 4: Conclusions

This study represents the first detailed investigation of within-river occurrences of introduced brown trout in eastern Newfoundland and confirms that brown trout have successfully invaded the Renews River watershed (this system was not originally stocked with brown trout). Brown trout were captured throughout the watershed, including all three river sections and in the estuary. In addition, this study documented the first known case of invading brown trout establishing resident populations, though the majority of individuals remain anadromous. Two YOY brown trout offspring of a resident fish were captured (one is MS and one in US); evidence that some invading adult brown trout adopted a non-migratory (resident) strategy in this watershed. This study also represents the first detailed investigation of seasonal estuary use by Atlantic salmon and brown trout, when both species are present, in North American waters (where Atlantic salmon is native and brown trout are introduced). Results show that estuary habitat use is an interactive function of species, life stage, and time of year. Furthermore, this is the first attempt to investigate the seasonal estuary use by introduced North American brown trout. Finally, the results confirm that LA-ICP-MS "drilling" or retrospective analysis is an effective means of extracting information on growth history and can identify YOY offspring of anadromous and resident fish.

The establishment of brown trout in the Renews River watershed has the potential to impact populations of native Atlantic salmon. Salmon stocks in eastern Canada have been in decline since the 1970s and have yet to recover (DFO). The finding of nearly year-round presence of adult brown trout in the estuary presents a potentially serious threat to the recovery and sustainability of Atlantic salmon populations. Brown trout are a key predator to salmon smolt during seaward migrations through estuary and coastal habitats (Koed et al. 2006; Larsson 1985). COSEWIC has recently recommended that southern Newfoundland populations of Atlantic salmon be listed as threatened (COSEWIC 2010), further highlighting the concern for Atlantic salmon populations in general. Regulatory agencies should consider this important finding and its potential consequences during efforts to aid the recovery of Atlantic salmon stocks in eastern Canada.

Species distribution patterns were analogous to those observed elsewhere in North America and in Europe; brown trout were more abundant in downstream and midstream habitats, while brook charr were more abundant in upstream habitats (including tributaries). Brown trout and brook charr did not co-evolve (brown trout are native to Eurasia, while brook charr are native to North America); therefore, interactions between these two species are the result of introductions into each fish's native habitat. Current research suggests that niche characteristics explain these patterns, whereby introduced brook charr colonize niche space (upstream headwater habitats) only marginally used by brown trout, whereas introduced brown trout colonized larger, more benign habitats further downstream that is less important for brook charr (Korsu et al. 2007). Results from our study support and contribute to this hypothesis, suggesting that brown trout are

indeed exploiting niche habitat in the river main stem and in downstream locations, while brook charr have retreated to their niche habitat in upstream and tributary locations. Therefore, a match between species niche requirements in its native range and habitat availability in the new environment may provide the basis for understanding invasion success (Korsu et al. 2010). This pattern was achieved in Newfoundland within approximately 125 years (perhaps much shorter as it is not known when brown trout invaded the Renews watershed) and this is the first known report on species distribution patters from local (i.e., watersheds along Newfoundland's southern shore) systems. Although brown trout invasion is occurring at a modest 4km/yr (Westley and Fleming 2011), and that minimal mixing between watersheds was occurring, at least from other systems into the Renews River watershed (Veinott et al.), the finding that local species distribution patterns mirror those in Europe after approximately 125 yrs is alarming. Regulatory agencies should consider this important finding and its potential consequences during efforts to manage native brook charr populations in eastern Canada.

An interesting observation was that brown trout have successfully invaded the Renews River watershed in spite of potential resistance from established native populations of Atlantic salmon and brook charr, which may present biotic resistance to invading brown trout (Moyle & Light 1996). The Renews River watershed is also relatively pristine, with little known anthropogenic disturbance; a characteristic not associated with successful invasions (Fausch et al. 2009). In addition, the Renews River watershed was not originally stocked with brown trout, and stocking efforts in the vicinity of St John's (approx. 90 km away) ceased in the early 20th century (Hustins 2007); removing continuous pressure on native species and nullifying a numeric advantage. In spite of these potential resistance factors, brown trout have successfully invaded and are now present throughout much of this system. Furthermore, they exploit anadromy and subsequent straying as a mechanism for continuing successful invasions. Regulatory agencies should consider this important finding and its potential consequences during efforts to aid the recovery of Atlantic salmon stocks and manage native brook charr populations in eastern Canada.

LA-ICP-MS "drilling" or retrospective analysis is an effective means of extracting information on growth history and can clearly distinguish between freshwater and marine environments. A major concern when using retrospective analyses of otoliths is that chemical sampling would not be confined to just core material (Chittaro et al. 2006). However, our results verify that this technique is effective for the purposes of identifying YOY offspring of anadromous and resident fish. The essential assumption in this study, and in the majority of studies using otolith chemistry, is that once otolith material is deposited it is permanently fixed (Kalish 1990; Rieman et al. 1994); the otolith core is not influenced by freshwater chemistry post emergence (Elsdon & Gillanders 2005). There is evidence suggesting that post-depositional change is indeed possible (Chittaro et al. 2006; Milton and Chenery 1998). However, our results confirm that the Sr88 concentration at the otolith core was not a function of the environment the individuals were collected from. Our conclusions are supported by new research suggesting that, although there is some chemical instability within the otolith core (i.e., manganese and magnesium),

chemical signatures for strontium (which was the element using in this study) appear stable (Veinott et al; unpublished data).

This study generated some limitations that should be considered when interpreting the results. In Chapter 2, the second hypothesis, which was that offspring of anadromous fish are of higher quality (i.e., bigger) than offspring of resident fish could not be tested; YOY from each species were largely offspring of migratory or resident fish, making within-species comparisons impossible. Although the sampling methodology was robust, available habitat for YOY fish and accessibility were also considered when fish sample sites were selected. Post emergence and end of growing season were assumed to be June and September, respectively; electrofishing is regulated to between June 15 and September 15 annually. Although emergence times and length of growing seasons can vary spatially and temporally, this assumption is generally supported by existing research (e.g., Klemetsen et al. 2003; Ohlund et al. 2008). Field sampling was conducted during one growth season (summer) only, and so the results reflect conditions for this time period and cohort. Despite considerable sampling effort, few brook charr were captured in downstream river sections, while few brown trout were captured in upstream reaches. In Chapter 3, field sampling was conducted during one calendar year only, and so the results reflect conditions for this time period. How effective sampling methods were at catching fish may not be known. However, using standard sampling methods and a robust approach, this study should accurately represent major trends, but precision for small scale interpretation is poor. The absence of captured fish over multiple sampling events (sets, sites, days, and tide cycles) has been interpreted as meaning they are indeed absent from the estuary. Temporary ice cover made sampling impossible during one tide cycle in December 2009, as well as January and February 2010; therefore, catch results during these months reflect reduced sampling effort.

In conclusion, brown trout in Newfoundland provided a unique and rewarding opportunity to investigate the successful invasion of an introduced non-native species and the impacts on native salmonids. The independent studies in Chapter 2 and 3 produced important insights and contributions that will support conservation efforts of Newfoundland's native populations of Atlantic salmon and brook charr. Furthermore, these studies revealed important contributions to our current understanding of why potential invasive species succeed or fail. Finally, confirming LA-ICP-MS laser "drilling" as an effective technique for extracting information on growth history should open the door for more diverse and complex studies. Brown trout in Newfoundland is an important resource (e.g., sport fishing and food source), yet pose a potentially serious threat to native Atlantic salmon and brook charr populations and are difficult to manage. Perhaps, the critical question is whether brown trout in Newfoundland should be managed as a sport fish or an invasive species.

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